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VARIABILITY IN THE PEA-WILT ORGANISM, FUSARIUM ORTHOCERAS VAR. PISI[1]

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INTRODUCTION

The occurrence of host specialization within species of parasitic fungi is widespread, being encountered frequently among the Basidiomycetes, Ascomycetes, and Fungi Imperfecti. In certain of the vascular *Fusaria* specialized strains of the parasite possessing different pathogenic capacities have been noted. Broadfoot (*3*)[3] described nine physiologic forms of the flax-wilt organism, *F. lini* Bolley, on the basis of various degrees of resistance recognized in four different flax varieties. The cotton-wilt fungus, *F. vasinfectum* Atk., was shown by Fahmy (*8*) to comprise three strains, depending upon whether the source of the fungus was Egypt, India, or North America. In a study of 24 collections of the tomato-wilt pathogene, *F. lycopersici* Sacc., White (*20*) was able to relegate all strains to two general classes on the basis of pathogenicity. These two groups were exemplified by the type A and B strains further studied for pathogenic and cultural differences by Haymaker (*11*). The A type was found consistently more pathogenic than the B type, but the difference proved to be a matter of degree rather than of kind, for the variations in strain virulence were uniform on all host varieties tested. Blank[4] encountered no selective pathogenicity or consistent variation in the degree of virulence of 11 collections of the cabbage-yellows parasite, *F. conglutinans* Wr., when applied to various subspecies of *Brassica oleracea* L.

In the pea-wilt disease, control is founded upon the use of resistant varieties. It is clear, therefore, that a survey of geographic isolations of the wilt fungus, *Fusarium orthoceras* App. & Wr. var. *pisi* Linford, is basic to the further development and use of wilt-resistant pea varieties. With this in mind, a study was made to determine the constancy in selective pathogenicity, and other characters, of the fungus.

PATHOGENICITY OF STRAINS OF THE PEA-WILT FUSARIUM

EXPERIMENTAL METHODS

A preliminary field trial was conducted in 1931 to determine whether a given set of pea varieties would react differently to naturally infested wilt soils in the different pea sections of Wisconsin. No evidence was found that pointed unmistakably to host specialization. A

[1] Received for publication Sept. 22, 1932; issued August, 1933.

[2] The author acknowledges his indebtedness to Dr. J. C. Walker for the valuable suggestions offered during the course of these investigations and for aid in preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 87.

[4] BLANK, L. M. A STUDY OF VARIATION IN THE CABBAGE YELLOWS ORGANISM, FUSARIUM CONGLUTINANS WR. Unpublished thesis, Univ. Wis. 1930.

more comprehensive study in the greenhouse was then undertaken in which cultures of the fungus derived from all available sources were employed.

For the sake of convenience, all isolations of the pea-wilt *Fusarium* studied from the standpoint of differences either in respect to pathogenicity or cultural characters will be termed "strains." In table 1 the regional origin of the fungus strain is indicated in each case by the numeral of the strain symbol, and isolants from the same region by the small letter following. Two or three isolations from each

TABLE 1.—*Source of Fusarium orthoceras var. pisi cultures used in the accompanying strain studies*

Strain of wilt	Regional source of strain		Material source	Pea-variety source	Date of isolation
	State	Locality			
1a	Washington	Fairfield	(a)	(a)	1929.
1b	do	do	Soil	Perfection	Winter, 1930.
2a	Idaho	St. Anthony	Plant specimens	Miscellaneous	Summer, 1930.
2b	do	Bonner's Ferry	do	Perfection	Summer, 1931.
2c	do	do	do	Alaska	Do.
3a	Montana	Bozeman	do	Perfection	Do.
4a	Wisconsin	Eau Claire	do	do	Summer, 1930.
4b	do	Waupun	do	do	Summer, 1929.
4c	do	Templeton	do	do	Summer, 1930.
5a	Illinois	Rochelle	Soil	do	Winter, 1930.
5b	do	do	do	do	Do.
6a	Indiana	Wabash	Plant specimens	Alaska	Summer, 1931.
7a	Pennsylvania	Hanover	do	Miscellaneous	Do.
7b	Maryland	Silver Run	do	do	Do.
7c	do	Westminster	do	do	Do.

[a] Culture WSC 15 received from L. K. Jones.

geographic area were selected exhibiting the range of culture type expressed by all isolations from that area. These 15 strains collectively are considered as representative of the pea-wilt fungus as it occurs throughout the pea-growing areas of the United States from Washington to Maryland (fig. 1).[5]

The cultures were purified by repeated transfer on agar plates prior to multiplication upon corn-meal sand. Single-spore cultures were not used because of the possibility of eliminating some potentialities of the original culture and because of the large number of such isolates that would be necessary in order to represent fairly the fungal range in each locality. By using gross cultures of one or more types a good wilt-fungus cross section of a given district could be obtained without the large numbers that would prohibit study of the strains in an experimental unit. Any strain differences observed as a result of these studies might then be further analyzed through the use of monoconidial lines of the fungus.

In the preparation of inoculum, 1-quart Mason jars half filled with a corn-meal sand medium were steam-sterilized and bits of the agar cultures introduced, 8 inoculations being made in this manner from each of the 15 strains. Eleven days later the contents of each jar were incorporated in enough virgin soil of a sandy-loam type to fill a 6-inch pot, and subsequently these were planted to 8 standard pea varieties, 4 of which were susceptible, and 4 resistant to wilt. In

[5] Since the completion of this manuscript the writer has found the pea-wilt fungus present also in California.

this set-up each strain could be tested simultaneously with 8 pea varieties, and by planting all the pots at one time a direct comparison of the host-parasite reactions of all strains of the fungus and of all pea varieties became possible. This procedure permitted a very heavy and uniform soil inoculation, insuring rapid wilting. Uninoculated check pots occupied the center row for the entire length of the greenhouse bench. Cross inoculation between pots was not a consideration in the experiment because, as has been shown elsewhere (*19*), appreciable quantities of soil inoculum must be transferred in order to function in disease production before three successive croppings to peas. Moreover, such high concentrations of inoculum were used that any effect of even a deliberate soil transfer would be overshadowed by the

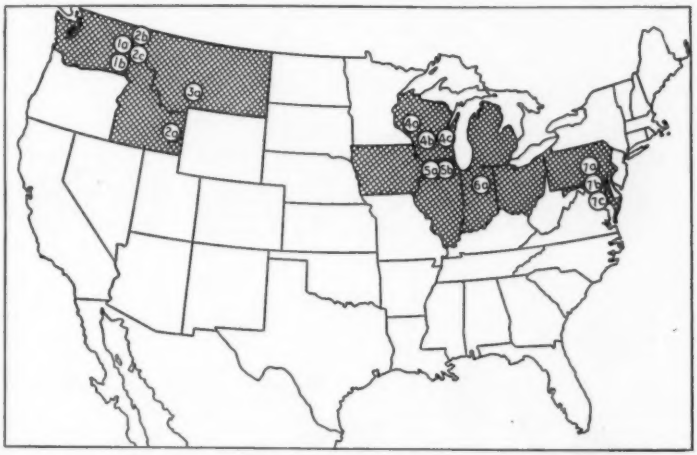

FIGURE 1.—Geographic distribution of the pea-wilt fungus as it occurs in the United States (*18*). Cross-hatching indicates the States in which the fungus is known to exist, while the circles designate the origin and number of the strains used in connection with the studies recorded here. The fungus was isolated from specimens sent in from Iowa during the summer of 1932.

reaction to the mass culture. The pots were used for only three plantings, and the check row was watched throughout the trials for any evidence of disease.

RESULTS OF PATHOGENICITY TRIALS

Data obtained from two consecutive plantings, series 1 and 2, of these soil inoculations are shown in tables 2 and 3. They show a marked uniformity of disease response dependent upon the pea variety used, regardless of the fungus strain employed. The two discontinuous classes of pea plants in respect to wilt resistance, classified in an earlier study (*17*) and here maintained when subjected to a wide collection of pathogenic wilt strains, are illustrated in figure 2, A. It will be observed that all the susceptible plants are completely dead and that all the resistant varieties are healthy. A few plants of susceptible Alaska still remain standing, but only one remained at the conclusion of the series. This stock of Alaska was found to have a scattering of wilt-resistant seed in it, not an uncommon experience with the Alaska variety. The percentages of wilt in

FIGURE 2.—A, A comparison of resistant and susceptible varieties from series 1 (table 2) 26 days after planting. All the pots shown here were inoculated with the fungus strain 7b from Maryland. Resistant (R) and susceptible (S) varieties are alternated as follows: *a*, Green Admiral (R); *b*, susceptible Alaska (S); *c*, resistant Alaska (R); *d*, Ashford (S); *e*, Alderman (R); *f*, Thomas Laxton (S); *g*, Bruce (R); *h*, Perfection (S). The plants of susceptible Alaska still standing in the pohtograph all wilted later except one which proved to be a resistant plant. B, Classification of wilt symptoms used in strain virulence studies: *a*, Healthy plant; *b*, diseased plant in early stages of wilt, showing stunting and recurving of the stipules and leaflets, at which stage the color is usually yellowish green; *c*, diseased plant completely wilted with all the foliage dead and shriveled although the stem is still green and turgid.

the susceptible varieties was smaller as a rule in the first series than in the second. The reason lies in the fact that during the first series of experiments growing conditions were unusually poor, the days were approaching their minimum in point of daylight, and sunshine was almost a rarity. Artificial light was employed but only with partial success. When final readings were taken, 7 weeks after planting, wilting was not complete in all cases, and some plants were collapsing as a result of spindly growth.

TABLE 2.—*Test of pea varieties susceptible and resistant to wilt against 15 different strain inoculations obtained from 7 pea-growing regions of the United States extending from Washington to Maryland*

SUSCEPTIBLE

Strain of wilt	Susceptible Alaska				Ashford			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*
1a	26	(a)	25	(a)	22	(a)	25	(a)
1b	22	86	26	100	23	100	26	100
Control	26	0	25	0	23	0	25	0
2a	26	81	24	96	21	86	29	100
2b	25	84	26	100	22	100	28	100
2c	25	80	25	96	22	100	25	100
3a	35	63	24	92	33	91	33	100
Control	31	0	25	0	27	0	25	0
4a	35	91	21	100	36	100	26	100
4b	34	62	25	92	29	96	24	100
4c	38	79	23	91	26	96	20	100
5a	42	83	22	96	30	100	28	100
5b	33	82	27	100	35	97	29	100
Control	39	0	25	0	24	0	25	0
6a	42	83	28	100	34	100	31	100
7a	40	100	27	93	36	100	28	100
7b	46	96	28	96	35	100	33	100
7c	40	95	25	100	31	77	26	100
Control	31	0	25	0	27	0	25	0

Strain of wilt	Thomas Laxton				Perfection			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*
1a	24	(a)	28	(a)	19	(a)	24	(a)
1b	25	88	29	100	26	100	26	100
Control	21	0	25	0	23	0	25	0
2a	20	65	27	100	27	89	30	100
2b	25	76	29	100	25	100	27	100
2c	24	100	26	100	28	100	29	100
3a	27	81	24	100	28	96	27	100
Control	26	0	25	0	25	0	25	0
4a	27	100	29	100	26	100	26	100
4b	28	100	33	100	29	86	28	100
4c	35	91	31	100	27	96	25	100
5a	37	100	29	100	29	100	28	100
5b	38	100	26	100	30	100	31	100
Control	29	0	25	0	32	0	25	0
6a	32	87	29	100	31	97	28	100
7a	35	91	30	100	24	100	28	100
7b	36	100	28	100	40	100	29	100
7c	35	97	32	100	38	87	34	100
Control	35	0	25	0	37	0	25	0

See footnotes at end of table.

TABLE 2.—*Test of pea varieties susceptible and resistant to wilt against 15 different strain inoculations obtained from 7 pea-growing regions of the United States extending from Washington to Maryland*—Continued

RESISTANT

Strain of wilt	Green Admiral				Resistant Alaska			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*
1a	20	0	24	0	25	0	26	0
1b	20	0	27	0	25	0	27	0
Control	22	0	26	0	25	0	24	0
2a	21	0	31	0	24	0	28	0
2b	21	0	32	0	21	0	33	0
2c	20	0	25	0	15	[b] 0	30	0
3a	27	0	29	0	30	0	27	0
Control	31	0	25	0	30	0	25	0
4a	30	0	24	0	34	0	29	0
4b	32	0	27	0	33	0	33	0
4c	39	0	28	0	30	0	32	0
5a	33	0	28	[b] 0	35	0	30	0
5b	33	0	31	[b] 0	35	[b] 0	28	0
Control	27	0	25	0	37	0	25	0
6a	31	0	29	0	40	0	26	0
7a	29	0	28	[b] 0	40	0	24	0
7b	33	3	32	3	40	2	31	[b] 0
7c	31	0	27	[b] 0	37	0	30	0
Control	29	0	29	0	40	0	28	0

Strain of wilt	Alderman				Bruce			
	Series 1		Series 2		Series 1		Series 2	
	Plants	Wilted	Plants	Wilted	Plants	Wilted	Plants	Wilted
	Number	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*	*Number*	*Per-cent*
1a	20	0	26	0	25	0	27	0
1b	25	0	23	0	27	4	26	4
Control	25	0	25	0	25	0	25	0
2a	25	0	29	0	26	0	29	0
2b	25	0	28	0	28	0	28	0
2c	25	0	30	0	29	[b] 0	30	0
3a	25	0	32	0	31	0	29	0
Control	25	0	25	0	25	0	25	0
4a	32	0	28	0	29	0	29	0
4b	35	0	33	0	31	0	31	0
4c	34	0	30	0	27	0	32	0
5a	32	0	34	0	32	[b] 0	30	3
5b	30	0	27	0	31	[b] 0	33	9
Control	25	0	25	0	25	0	29	0
6a	32	0	29	0	27	0	30	0
7a	35	0	30	0	33	[b] 0	28	[b] 0
7b	30	0	41	0	30	[b] 0	31	6
7c	40	0	33	0	34	0	29	0
Control	38	0	28	0	30	0	27	0

[a] Incomplete. Owing to the slow rate of wilting obtained with this strain the percentage of wilt at the completion of both series was not comparable to other data in the table, so was not recorded.

[b] Leaf symptoms, usually on 1 to 5 plants, suggested wilt, but culture of the plants on agar did not yield the fungus.

Upon the basis of these data it appears that the programs of selection and breeding for pea varieties resistant to *Fusarium orthoceras* var. *pisi* are built upon the relatively firm foundation of (1) the stable nature of the wilt pathogene as regards kind of pathogenicity, and (2) the hereditary nature of resistance, carried by a single dominant

factor (*17*). These facts make possible a simple and very effective means of pea-wilt control. On the other hand, however, the possibility remains that a differentially pathogenic strain of the parasite may sometime appear, or may even now occur undetected. The wide differences between strains in respect to other fungal characters emphasizes this possibility.

TABLE 3.—*Summary of resistance to 14 strains of the Fusarium wilt fungus of 8 pea varieties planted in series 2 (table 2) on artificially inoculated soil, as compared to a planting from the same seed stocks on naturally infested Waupun wilt soil*

Pea variety	Artificially inoculated soil		Naturally infested Waupun soil	
	Plants	Resistance	Plants	Resistance
	Number [a]	*Percent* [b]	*Number*	*Percent*
Green Admiral	398	90.7	91	100
Resistant Alaska	408	100	115	99.2
Alderman	427	100	52	100
Bruce	415	98.4	124	100
Susceptible Alaska	356	3.3	122	8.9
Ashford	395	0	93	2.1
Thomas Laxton	399	0	106	1.9
Perfection	396	0	127	.8

[a] These numbers do not include those planted on the soils inoculated with fungus 1a.
[b] Based upon the number of instances of susceptibility or resistance encountered throughout the experiment with each variety.

COMPARATIVE VIRULENCE OF STRAINS

Although no evidence was obtained in the course of these investigations to suggest that a differential in host specialization between strains exists, it appeared that the strains varied somewhat in respect to virulence or degree of pathogenicity. Records on the appearance of symptoms and dates at which complete wilting or death of the plants occurred showed significant differences in this regard. A comparison was possible, owing to the fact that all pots in the experiment had received identical treatment. In the second series, those pots in which the resistant varieties had grown during the first series, were planted with susceptible seed and vice versa. By the end of the second series it was expected any minor differences in inoculum concentration of the soils had become stabilized through the intensive culture of peas. An additional planting at this time was planned in order to record differences in strains in respect to rate of wilting using only one variety of pea, and to determine whether these differences corresponded with those noted in the previous series.

The pots were planted with Perfection seed, and the total stand in each case was taken. Every 2 days counts were made of the number of plants showing symptoms, and of the number dead, i.e., those plants whose foliage was entirely wilted and shriveled. These two symptom classes, distinct in themselves, are shown in figure 2, B. In table 4 are computed the percentages of plants diseased, and dead, at 2-day intervals following planting. It is seen that the variation in appearance of symptoms ranges from 14 days after planting in several strains to 28 days in the case of 1a. Similarly, complete wilting is an index of the relative degree of strain pathogenicity. In the last column the strains are rated in respect to virulence both as indexed by

symptom appearance and complete wilting. At the top of the column are the most virulent strains, and at the bottom the least virulent ones. By plotting the percentages, either of diseased or of dead plants against time in days, curves representing the relative virulence of each strain were found to parallel each other closely in form and type and to fall into four general groups. Since parallelism of the curves was most pronounced in the region of the graph representing 50 percent killing of plants, a comparative rating of strains was made at this point. Accordingly, the value in days for each curve as it crossed the 50 percent line was read on the graph, and these figures were used in constructing figure 3. All strains are seen to fall into four groups, the first three of which are separated by approximately 3-day intervals, and a fourth group represented by strain 1a alone. Observations in both series 1 and 2, in conjunction with this experiment,

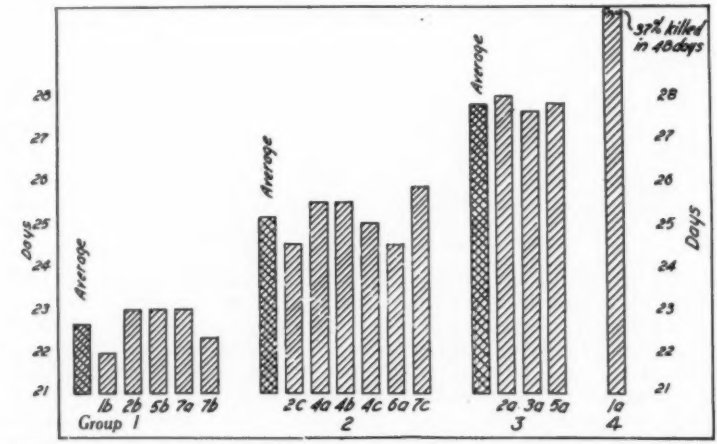

FIGURE 3.—Comparison of the number of days required for each wilt strain to kill 50 percent of the plants.

support the arbitrary classification portrayed here. In any event it is clear that differences occur between strains. Strains 1b and 7b in all inoculation work have always been in the most virulent class. Strain 1a has been the most divergent of all the strains studied. At the close of the experiment 7 weeks after planting only 37 percent of the plants had been killed. Reisolation of the strain from these plants and reinoculation of soil therewith have given the same type of results, namely, that in spite of a heavy soil inoculation wilting may be completed only in two or three times the interval observed in the case of other strains. Cultural characters also diverge somewhat from the general type range of the strain collection taken as a whole.

In order to compare the strains under more closely similar conditions the fungus was reisolated from diseased plants on soils 1a, 2a, 2b, 2c, 4c, and 7c, these representing the four groups shown in figure 3. Isolations were all made at the same time, multiplied on the same weight of corn-meal sand, and equal volumes of soil inoculated and planted the same day. In this manner each strain was recovered in a pathogenic state, and maintained in artificial culture for the same

period of multiplication before being returned to a new lot of thoroughly mixed untreated virgin soil. The interval between the recovery of the strain from diseased plants and reincorporation in the soil, was 18 days. The relative rate of wilting obtained was of the same order as indicated in figure 3, except that 2a took a position between 2b and the group 2c, 4c, and 7c.

TABLE 4.—*Rate of appearance of wilt symptoms, and of death, as expressed in percentages of affected Perfection pea plants during a period of days, for different cultural strains of the wilt fungus*

Strain of wilt	Plants	14 days		16 days		18 days		20 days		22 days		24 days		26 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1a	140	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1b	121	4.1	0	97	0	98.4	0	100	36.4	100	49.6	100	79.3	100	96
2a	148	0	0	0	0	4.1	0	16.2	0	47.3	0	69	7.4	90	21
2b	184	2.8	0	90.2	0	92	0	97.3	15.2	100	34.2	100	74	100	96.2
2c	46	0	0	2.2	0	74	0	75	4.3	85	8.7	100	32.6	100	87
3a	211	0	0	0	0	2.4	0	10.9	0.5	64	1.4	84	2.8	96	10.9
4a	189	0	0	1.1	0	2.6	0	93	0	96	1.1	98	7.9	100	73
4b	247	0	0	2.0	0	3.6	0	94	0	96	0.4	98	8.9	100	70
4c	225	23.2	0	89	0	90	0	91	4.5	93	5.8	96	9.3	99	84
5a	56	0	0	0	0	3.6	0	16	0	53.6	0	89	3.6	100	14.3
5b	268	0	0	2.9	0	91	0	100	8.6	100	28	100	78	100	93
6a	201	2.5	0	85	0	90	0	91	6.9	91	11	97	40	99	67
7a	68	1.5	0	85	0	90	0	94	13.2	94	30.9	98.5	72	98.5	89.8
7b	76	92	0	96	0	96	0	100	10.5	100	42	100	77.7	100	97.5
7c	279	0	0	0	0	1.1	0	12.2	0	54	0	65	35.8	87	51

Strain of wilt	Plants	28 days		30 days		32 days		40 days		48 days		Order of rating of wilt strains [a]	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	By disease	By death
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		
1a	140	1.4	0	3.6	0	14.3	0	65.8	5.7	82	37.1	7b	7b
1b	121	100	100	.....	.....	100	100	100	100	100	100	1b	1b
2a	148	100	57.4	100	68	100	82	100	100	100	100	7a	7a
2b	184	100	99	.....	.....	100	100	100	100	100	100	4c	2b
2c	46	100	100	.....	.....	100	100	100	100	100	100	2b	5b
3a	211	100	54	.....	.....	100	86	100	100	100	100	6a	6a
4a	189	100	95	.....	.....	100	100	100	100	100	100	5b	2c
4b	247	100	95	.....	.....	100	99	100	100	100	100	2c	4c
4c	225	100	93	.....	.....	100	98	100	100	100	100	4b	4b
5a	56	100	57	.....	.....	100	77	100	100	100	100	4a	4a
5b	268	100	98	.....	.....	100	100	100	100	100	100	3a	7c
6a	201	100	91	.....	.....	100	96	100	100	100	100	2a	3a
7a	68	100	95.6	.....	.....	100	100	100	100	100	100	7c	2a
7b	76	100	98.8	.....	.....	100	100	100	100	100	100	5a	5a
7c	279	100	66	.....	.....	100	86	100	100	100	100	1a	1a

[a] Listed in descending order of virulence.

A comparison was next made with single-spore cultures from several strains, as follows: 1a5, 2a2, 4b1, 4c4, 5b3, and 7b5. They were used in duplicate, multiplied on weighed amounts of inoculum, and mixed into the soil in the same manner as before. The single-spore lines of 5b and 7b fell into the most virulent group, 4b and 4c were of inter-

mediate virulence, and 1a was the least virulent. Strain 2a again approached in pathogenicity group 1.

The results up to this point argued for a general constancy of virulence for a given strain culture. However, the experience with strain 2a, indicating that different cultures of the same strain source might vary in degree of pathogenicity, and the additional fact that isolations from the same geographic regions may exhibit all the degrees of virulence illustrated in figure 3, suggested the possibility that a given strain might be made to yield forms both higher and lower in virulence than the original. In order to test this possibility a susceptible variety of peas was grown upon naturally infested Waupun soil, the original source of strain 4b. Thirty-five resulting wilted plants were cultured and 35 isolations of the wilt fungus obtained. Single-plate colonies were made of each isolation for observation in respect to purity, and they were then transferred, one colony per plate, to 5 percent potato-dextrose agar, a medium found favorable for the separation of variants. Five of the cultures were conspicuous in the production of cultural types; these were built up on corn-meal sand and aliquots of virgin soil inoculated and planted. The results are shown in figure 4. Comparison with figure 3 is not possible, because this series was grown at a time when for 2 or 3 weeks there was but little sunshine and wilting proceeded slowly. However, interrelationships were not altered, and two inoculations with the original stock culture 4b were run for a standard.

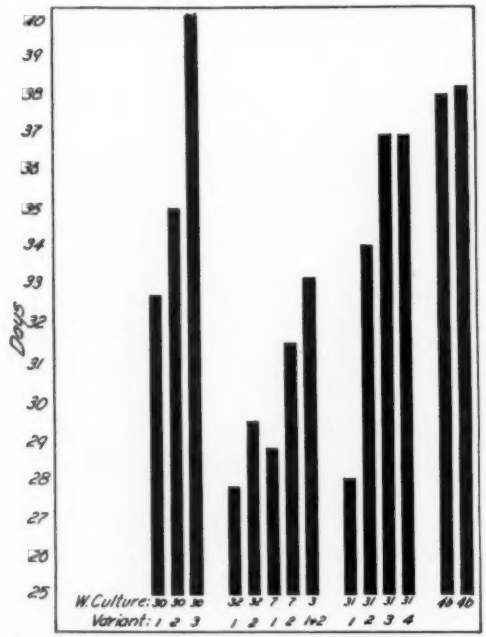

FIGURE 4.—Comparison of the number of days required to obtain 50 percent killing of plants with segment cultures (variants) of several isolations of the wilt fungus from the same source.

It is realized that the use of segment cultures from a gross isolation of the fungus in an endeavor to demonstrate variability within a single colony of the fungus is open to the objection that the original isolation may have contained two or more strains. It became essential, therefore, in considering the potentialities of a single thallus in producing variants with different capabilities in regard to virulence, to run an experiment with single-spore cultures and compare the virulence of variants obtained from them, both in relation to each other and to the parent culture. Culture 4b was selected for this

purpose. The procedure consisted in placing several monoconidial lines of strain 4b upon a medium which readily permitted the detection of variants. Mycelial transfers of the sectors were then made from time to time. Two of these variants are illustrated in figure 5. In this way a collection of variants was obtained, all of which originated in single-spore cultures of one strain. Several of the more stable of these dissociants were then multiplied on equal weights of corn-meal sand and incorporated in aliquots of soil under identical conditions. Essentially the same relative virulence was obtained as with segments of gross cultures of the strain. The dissociants possessed a virulence higher, lower, or the same as the monoconidial parent culture. These relative differences are shown in figure 6.

The foregoing experiments established the fact that within the fungus causing the Fusarium wilt of pea there occur strains differing in degree of pathogenicity. The differences in virulence obtained were

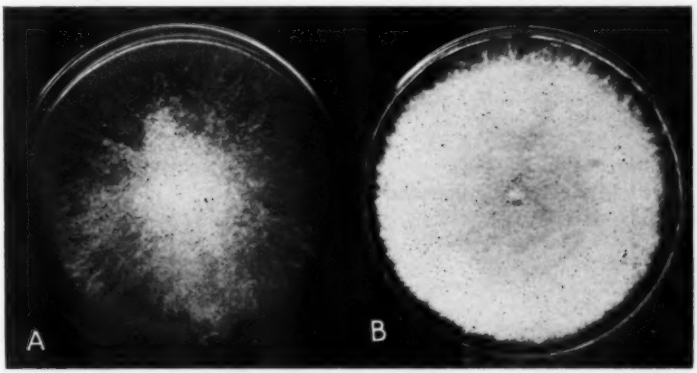

FIGURE 5.—Two segment cultures from the same parent single-spore colony (4b5). A produced a deep red pigmentation in the medium; B exhibits more abundant aerial mycelium, more rapid radial growth, and lacks pigmentation.

not wide as a general rule, but they were clearly defined. It is, of course, not to be expected that a variable fungus, existing in a labile state, should exhibit fixed pathogenicity relationships, especially where fragment cultures representative of the most unstable condition of the fungus are compared.

Repeated inoculations with the same stock culture have yielded results all of the same order. For example, a dozen soil inoculations made over a period of a year with stock culture 4b have always exhibited an intermediate type of virulence, and the same is true of other strains. However, one geographic region or even a locality is not to be considered inhabited by a single strain type. Two widely divergent strains have been studied from the same pea region in Washington. Attempts to build up the virulence of the weaker strain (1a) by repeated reisolation and reinoculation have failed. The strains from Maryland on the opposite coast of the United States showed similar differences in degree of pathogenicity and so with other regions. Strains both more and less virulent than the original 4b culture were obtained from the same bed of Wisconsin soil, using monoconidial lines. Thus a locality or region may be dominated by

a strain type but not restricted to that type. This is to be expected in view of the dissociating nature of the fungus, and the fact that it is seed borne (*16*).

Duplicate inoculations of all trials have been practically identical in their pathogenesis, thereby vouching for the technic employed.

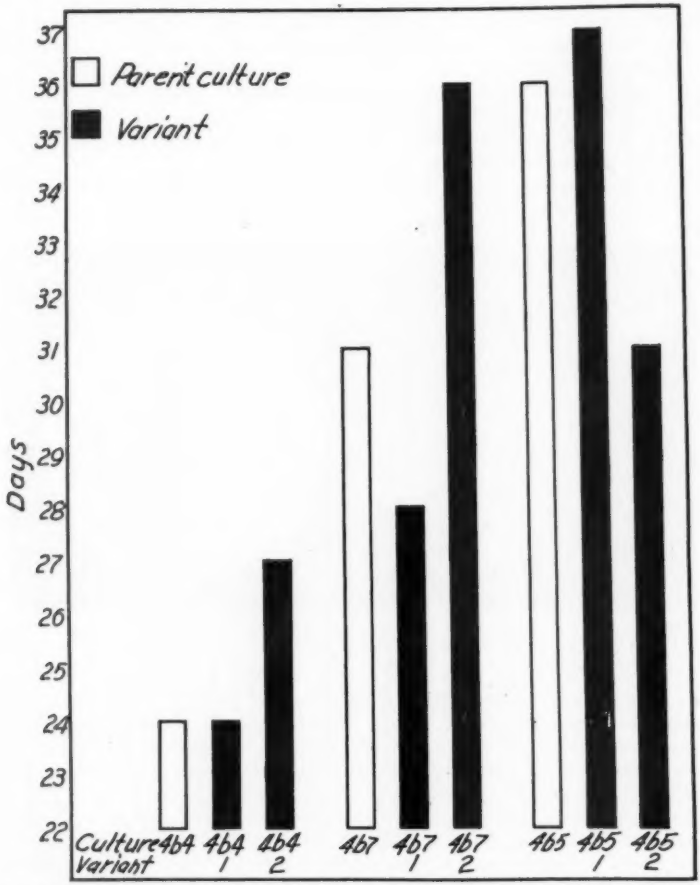

FIGURE 6.—Comparison of the number of days required to obtain a 50 percent killing of plants with segment cultures (variants) from monoconidial parent cultures of wilt strain 4b.

It has occurred to the writer that although equal amounts of cornmeal sand were inoculated with each strain, different growth rates upon the medium might result in different amounts of fungus being added to the soil, and that the greater virulence was a direct correlation of a higher concentration of fungus inoculum and not an expression of fungal potency. Cultural characteristics of the strains are discussed elsewhere; it suffices to state here that no direct correlation has been encountered between virulence and growth, in fact

some of the fastest and most profuse growers possess the least virulence. Correlation with other cultural characters are discussed later in this paper.

PHYSIOLOGY OF THE PEA-WILT FUSARIUM

In the original description of *Fusarium orthoceras* var. *pisi* it was noted that cultures of the fungus showed divergence among themselves especially in respect to pigmentation. In fact one agreed closely with *F. conglutinans*, the cabbage-yellows organism (*14*). Linford, however, reports no comparative study of different isolations. It was deemed opportune, therefore, to parallel the trials of comparative pathogenicities with some observations upon culture characters of the strains.

At the outset it was realized that the building up of a mass of data upon characters of growth in artificial culture could only have limited value in the absolute sense because of the inconstancy of colony type, even where single-spore lines of the fungus were employed. It was hoped, however, to bring out relative differences between strains which might be correlated with results from the pathogenicity trials.

The strains varied somewhat among themselves in respect to several cultural characters. Perhaps the most conspicuous of these was variability in the capacity for the production of color. Differences in type of mycelial growth, whether flat and sparse, or aerial and abundant, were encountered as well as differences in colony margin, rates of growth, staling, and sporulation. Because of the inconstancy of characters within a given stock culture of a strain, it seemed desirable, if not necessary, to confine the study to single-spore lines. Accordingly, a number of monoconidial lines were obtained for each strain. Most of these in each case were of the gross parent type,while others diverged from the original in colony characters. Representatives of the majority type (in every case these were also representative of the original culture) were used in the comparison of strains recorded in table 5. Reisolates from the pathogenicity trials, discussed earlier in this paper, conformed in general to these types, particularly in respect to pigmentation.

TABLE 5.—*Comparison of the cultural characters of single-spore lines of the 15 strains of the wilt fungus, grown in Petri dishes upon aliquots of 5 percent potato-dextrose agar*

[All data was taken 35 days following inoculation]

Strain of wilt	Sectors [a]	Aerial mycelium	Pigmentation of—	
			Medium	Hyphae
1a	−	Scant	White	White.
1b	+	Abundant	Auburn [b]	Do.
2a	+	Moderate	Dark Perilla purple	Maroon.
2b	+	do	Dark Corinthian purple	White.
2c	++	do	do	Do.
3a	+	do	Walnut brown	Do.
4a	+	do	Dark Perilla purple	Do.
4b	+	do	do	Do.
4c	−	Abundant	Ecru drab	Do.
5a	−	Moderate	Hessian brown	Gray-white.
5b	−	Abundant	Light pinkish cinnamon	White.
6a	−	Moderate	Dark Corinthian purple	Do.
7a	+	Abundant	Corinthian red purple	Do.
7b	+	do	Dark Corinthian purple	Do.
7c	+	Moderate	do	Do.

[a] (−) indicates no conspicuous sectoring, (+) slight, and (++) moderate.

[b] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

Six single-spore lines from 1a, 2a, 4b, 4c, 5b, and 7b, typifying the types most divergent as to cultural and virulent qualities, were selected for further comparisons. A study of these on modified Czapek's medium, using glucose and pectin, respectively, as sources of carbon, was made at five constant temperatures. The results are represented in figure 7. Since the curve for 4c so closely coincided with that for 4b no attempt was made to differentiate between them on the chart. Distinct differences in the rates of growth are illustrated between such

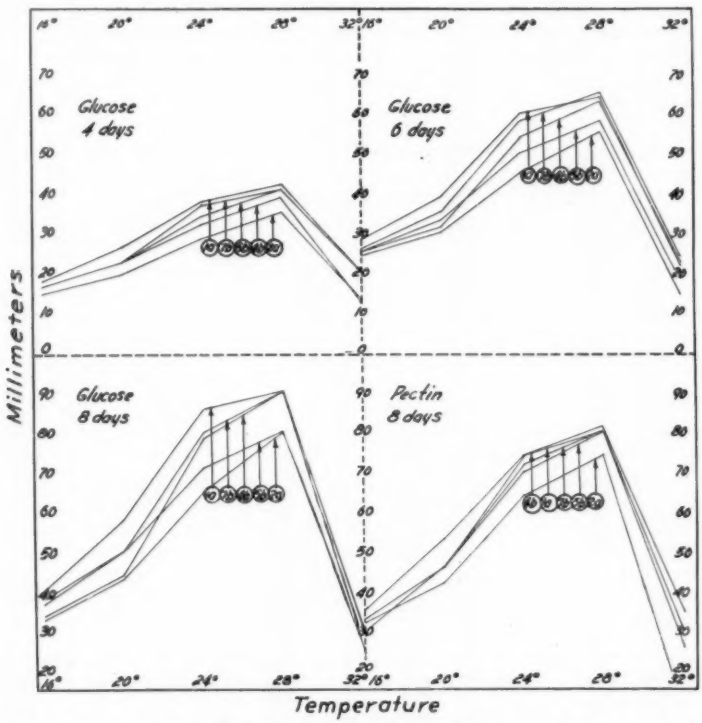

FIGURE 7.—Average colony diameters of a monoconidial line from each of the five strains of *Fusarium orthoceras* var. *pisi* indicated, at five constant temperatures. A modification of Czapek's medium was employed, using glucose and pectin, respectively, as sources of carbon.

extremes as 1a and 2a, depending upon the medium, but the temperature characteristics reported by Linford (*14*) in his original description of the fungus were found to hold in general for all types. As a result no wide differences in strain-disease relationships are to be expected in regard to temperature.

These six monoconidial lines and the organisms causing *Fusarium* wilts of aster (*F. conglutinans* var. *callistephi* Beach), beet (*F. conglutinans* var. *betae* Stewart), celery (*Fusarium* sp.), cowpea (*F. tracheiphilum* (E. F. S.) Wr.), and watermelon (*F. niveum* E. F. S.), were used in other comparative studies. The cabbage-yellows pathogene (*F. conglutinans* Wr.) was also included, but a portion of the results were discarded because of bacterial contamination. This fungus

however has in other series agreed closely in reaction with its varieties. A modified Czapek's medium was used.[5] To this a carbohydrate was added in sufficient quantity to give a final concentration of 5 percent. The solution of the carbohydrate was sterilized separately in the autoclave and added to the medium at the time the plates were poured. At the same time the reaction of the final medium was adjusted to pH 6 except in the case of xylose, xylan, and pectin. All Petri dishes were poured to an approximate depth of three fourths of a centimeter to eliminate the growth differences sometimes experienced on thin agar plates (*4*), and inoculated in duplicate. Comparable bits of aerial mycelium were taken for this purpose from cultures of the same age on the same medium and the series run at a temperature of 21.5° to 22.5° C. for a period of 5 weeks.

The very close similarity of duplicate cultures throughout the experiment served to emphasize the significance of strain and carbon-source differences. Within a week after the media were inoculated striking differences in pigmentation became apparent. Color as expressed in this series varied in three important respects, namely, (1) rate of appearance, (2) intensity, and (3) shade. Table 6 shows the relative intensity and rate of color formation at the end of 10 and 30 days. Based upon results up to the time the fungus growth had completely filled the Petri dish, the fungi could be separated into two distinct groups, the pigment producers and nonpigment types. At all times differences in relative intensity as well as in shade of color were apparent between strains of the pea-wilt *Fusarium*, for example 2a, 4b, and 7b.

TABLE 6.—*Comparison of the ability of certain vascular Fusaria to produce pigmentation upon a synthetic agar medium in 10 and 30 days, employing 5 percent concentrations of different carbon sources*

Fungus culture	Item	Color of medium [a] indicated					
		Xylose	Arabinose	Araban	Pectin	Galactose	Glucose
F. orthoceras var. *pisi* 1a5	10 days	0	0	0	0	1	0
	30 days	Trace.	1	Trace.	0	2	1
	Quality	202	7L1	8A5	0	56L12	6L12
F. orthoceras var. *pisi* 2b2	10 days	0	2	0	0	1	0
	30 days	1	3	1	0	1	2
	Quality	3H9	56L6	8J9	0	56L6	56L12
F. orthoceras var. *pisi* 4b1	10 days	0	0	0	0	0	1
	30 days	0	2	0	0	2	2
	Quality	0	6K11	0	0	7L4	56L12
F. orthoceras var. *pisi* 4c4	10 days	0	0	0	0	0	0
	30 days	1	2	0	0	1	1
	Quality	2D2	7L1	0	0	7L4	[b]
F. orthoceras var. *pisi* 5b3	10 days	0	0	0	0	0	0
	30 days	0	2	0	0	2	1
	Quality	3F10	6011	0	0	5J12	5J12
F. orthoceras var. *pisi* 7b5	10 days	0	2	1	0	1	2
	30 days	0	3	1	0	3	3
	Quality	2E8	56L12	8A5	0	56L6	56L6
F. conglutinans var. *callistephi*	10 days	0	0	0	0	0	0
	30 days	0	1	0	0	0	0
	Quality	0	5H9	0	0	0	0
F. conglutinans var. *betae*	10 days	0	0	0	0	0	0
	30 days	0	0	0	0	0	0
	Quality	0	0	0	0	0	0
Fusarium sp. causing celery wilt.	10 days	1	----------	0	0	2	1
	30 days	1	----------	Trace.	0	2	2
	Quality	----------	----------	----------	0	47L11	5R4
F. tracheiphilum	10 days	2	0	0	0	2	2
	30 days	2	1	0	0	2	2
	Quality	----------	46J5	0	0	47L11	56L8
F. niveum	10 days	0	----------	0	0	1	1
	30 days	0	----------	Trace.	0	1	1
	Quality	----------	----------	----------	0	46J7	46J7

[a] The numerals indicate a measure of relative intensity; for "quality" see Maerz, A., and Paul, M. R. (*15*).
[b] Lost.

[5] $MgSO_4$, 0.5 g; $K_2H_4PO_4$, 1 g; KCl, 0.5 g; $FeSO_4$, trace; asparagine, 1 g; agar, 20 g; H_2O, 1 liter.

TABLE 6.—*Comparison of the ability of certain vascular Fusaria to produce pigmentation upon a synthetic agar medium in 10 and 30 days employing 5 percent concentrations of different carbon sources*—Continued

Fungus culture	Item	Color of medium [a] indicated				
		Dextrin	Lactose	Saccharose	Maltose	Mannite
F. orthoceras var. *pisi* 1a5	10 days	0	0	0	1	0
	30 days	2	0	2	3	3
	Quality	56L12	0	6L12	56L12	56L12
F. orthoceras var. *pisi* 2b2	10 days	2	0	1	3	3
	30 days	4	Trace.	3	4	4
	Quality	56L6		56L12	56L6	56L6
F. orthoceras var. *pisi* 4b1	10 days	1	0	1	0	2
	30 days	3	0	2	2	3
	Quality	56L12	0	56L12	5J11	56L12
F. orthoceras var. *pisi* 4c4	10 days	0	0	0	0	0
	30 days	1	1	2	2	3
	Quality	6B11	5B8	7L6	7L1	56L12
F. orthoceras var. *pisi* 5b3	10 days	0	0	0	0	0
	30 days	1	0	2	Trace.	3
	Quality	6B11	0	6L11	4E10	7L4
F. orthoceras var. *pisi* 7b5	10 days	3	0	2	2	3
	30 days	4	Trace.	3	3	4
	Quality	56L6	6A10	56L12	56L12	56L6
F. conglutinans var. *callistephi*	10 days	0	0	0	0	0
	30 days	Trace.	0	0	1	3
	Quality	46D1	0	0	5H9	7L1
F. conglutinans var. *betae*	10 days	0	0	0	0	0
	30 days	0	0	Trace.	0	0
	Quality	0	0		0	0
usarium sp. causing celery wilt	10 days	3	0	2	1	3
	30 days	3	0	2	2	3
	Quality	56C8	0	5H4	5H4	(b)
F. tracheiphilum	10 days	3	0	3	1	1
	30 days	3	Trace.	3	2	3
	Quality	47L11	41D2	47L11	46J5	56C8
F. niveum	10 days	1	0	0	1	1
	30 days	1	Trace.	1	1	2
	Quality	46J7	46J11	46J7	46J7	(b)

[a] The numerals indicate a measure of relative intensity; for "quality" see Maerz, A., and Paul, M. R. (*15*).
[b] Lost.

An examination was made of coloration in the steles of wilted plants grown upon the different strain inoculations to see if any relation was borne out between pigment production on artificial media and that where natural vascular discoloration occurred in diseased plants. There seemed to be no rule in this respect for the plants examined, either as to intensity or quality of color.

Attempts were also made to correlate pigmentation and virulence, since culture studies exhibited such pronounced differences in respect to the former. Strain 1a, of lowest virulence, produced no pigment as a rule except after long periods of time on rich carbohydrate media; 7b, exhibiting as high virulence as any strain collected, vied with 2a in rapidity and intensity of pigmentation; 2a exhibited both low and high virulence in various tests, but the single-spore line used here proved to possess a virulence ranking with 7b; 4c, intermediate in degree of virulence, was a poor pigment former, whereas 4b of a similar virulence rating yielded pigment more rapidly than 4c or 1a, but slower than 2a or 7b; 5b failed most conspicuously in the attempted correlation, but both Illinois strains, 5a and 5b, were also markedly different from all the other strains of the fungus in the shade of color when pigmentation did occur. On these high-sugar media the color with all strains was usually of varying intensities of an orange to purple red, except for 5a and 5b which were on the order of a yellow to pink, light brown. When the strains are grouped into classes

according to rate of pigment formation, some manner of correlation between pigmentation and high virulence may be noted in a general way. However, pathogenicity trials in which color and noncolor variants of the same single-spore parent were compared, yielded no consistent data. The extremes in pigmentation of comparable cultures from a very dark almost-black maroon to pure white gave the impression at times that different species rather than strains of a single variety were being studied. All the intensities (though not shades) of pigmentation found between the vascular *Fusaria* cultured were surpassed by the range of intensities within *F. orthoceras* var. *pisi*.

It has been observed (*4*) that the pH value as well as a high carbohydrate content of the medium, may influence pigmentation. At the conclusion of this experiment a rough approximation of the pH value was obtained by applying a drop of indicator to the agar of the Petri dish. Where pigmentation prevented a direct reading in this manner, a small piece of white filter paper was first pressed upon the agar and the indicator placed upon the paper. With all six strains and on all carbon sources used except pectin, the final pH value was found to be in the neighborhood of 3 to 5. The pectin medium, on the other hand, although most acid to start with, ended in every case on the alkaline side with a pH value of about 8. No pigment was formed in this medium, the light-brown color imparted by the pectin persisting throughout. Whether pigmentation in the cases of strains 2a and 7b could be correlated with a more rapid development of acidity in the medium is not known inasmuch as the pH readings were taken only at the beginning and conclusion of the experiment.

The fungi other than *Fusarium orthoceras* var. *pisi* compared similarly among themselves and with the pea-wilt fungus in most respects. The parasites of cowpea and celery, and next, watermelon, produced more pigment than did the varieties of *F. conglutinans*, but no more than certain strains of the pea fungus. Pigmentation of these species tended to be more on the purple than on the red side. The pH values were also of the same order in most instances where read.

Measurements of radial growth were taken on all plates at 2-day intervals. Duplicate plates showed marked uniformity, for the most part, not varying more than 1 or 2 mm in diameter. Differences in rate of growth between some strains of the pea-wilt fungus were exaggerated on certain carbon sources, as, for instance, on galactose, while in other cases, exemplified by the pectin medium, the differences were small, not exceeding 10 mm of diameter growth. The relative rates of growth varied somewhat with the medium, but strains 1a and 7b were generally the most rapid in radial growth, while 2a and 4b were usually among the slowest. Greatest radial growths of any of the fungi studied were exhibited by the celery and cowpea parasites.

The relative amounts of growth often bore no correlation to radial development of the mycelium. Because of the lack of direct means of comparing cultures in this respect, an arbitrary standard of classification was set up through the use of plate colonies representative of 10 classes of growth occurring in the experiment. Although the basis for the 10 classes is largely an evaluation of abundance of aerial

mycelium, the density and other growth characters have also been taken into account. All colonies were compared in this manner after 10 days and at the end of 30 days. Inasmuch as the ratings were relatively constant throughout the period of growth, only those recorded after 30 days are given in table 7. Strains of the pea *Fusarium*, it is noted, produced the least growth as a rule on xylose, xylan, and arabinose, exhibited various degrees of preference for araban, galactose, glucose, dextrin and sucrose, and did uniformly well on pectin, lactose, maltose, and mannite. Figure 8, photographed at the time the observations in table 7 were recorded, shows all the fungi used in the experiments grown on a medium containing sucrose as the carbon source. Differences in growth between the fungi are evident, but these differences for the most part are no greater than those within the strain range of the pea-wilt fungus.

TABLE 7.—*Comparison of the relative amounts of growth produced by certain vascular Fusaria in 30 days upon a synthetic-agar medium employing 5 percent concentrations of different carbon sources*

[Three fourths of a centimeter thickness of agar was used in each plate and the temperatures maintained at 21.5° to 22.5° C.]

Fungus culture	Growth on indicated medium as evaluated by an arbitrary numerical standard											
	Xylose	Xylan	Arabinose	Araban	Pectin	Galactose	Glucose	Dextrin	Lactose	Sucrose	Maltose	Mannite
F. orthoceras var. *pisi* 1a5	1	----	2	8	9	5	2	7	9	2	7	5
F. orthoceras var. *pisi* 2a2	1	2	2	2	6	4	2	3	8	2	5	6
F. orthoceras var. *pisi* 4b1	2	----	6	8	7	7	4	7	7	4	5	7
F. orthoceras var. *pisi* 4c4	2	3	6	8	7	7	6	2	8	8	8	8
F. orthoceras var. *pisi* 5b3	1	2	6	3	7	8	8	6	7	8	7	8
F. orthoceras var. *pisi* 7b5	2	4	7	8	8	3	6	7	7	6	7	7
F. conglutinans var. *callistephi*	1	----	2	1	4	3	2	3	3	2	2	3
F. conglutinans var. *betae*	1	----	2	2	7	7	3	3	6	3	3	6
Fusarium sp. causing celery wilt	4	----	----	2	2	2	10	3	----	10	10	6
F. tracheiphilum	4	----	2	3	9	7	10	6	10	10	3	10
F. niveum	3	----	----	3	6	6	4	4	6	7	2	6

In comparing the growth characters the phenomenon of spiral colony development was observed in many cases, especially during the early stages of growth. Where present, the spiral was recorded as having either a clockwise or counterclockwise direction. This feature was particularly conspicuous upon lactose and xylan, and moderately so on mannite, pectin, galactose, and maltose. A record of the phenomenon in these cases is found in table 8.

TABLE 8.—*Occurrence and direction of spiral colony development of monoconidial lines of the pea-wilt fungus upon different carbon sources*

[C indicates clockwise and A counterclockwise growth]

Strain of wilt	Growth on type of medium indicated							
	Lactose	Xylan	Maltose	Galactose	Pectin	Mannite	Arabinose	Araban
1a5	C	----	----	A	C	C	C	C
2a2	C	C	C	A	C	C	C	C
4b1	C	----	C	A	----	C	A	----
4c4	C	C	C	A	C	C	C	C
5b3	C	C	C	----	C	----	----	C
7b5	----	C	C	A	C	C	A	----

There appears to be uniformity among these single-spore lines in regard to the direction of spiraling, but this uniformity was not observed in the various other fungal species and varieties in the experi-

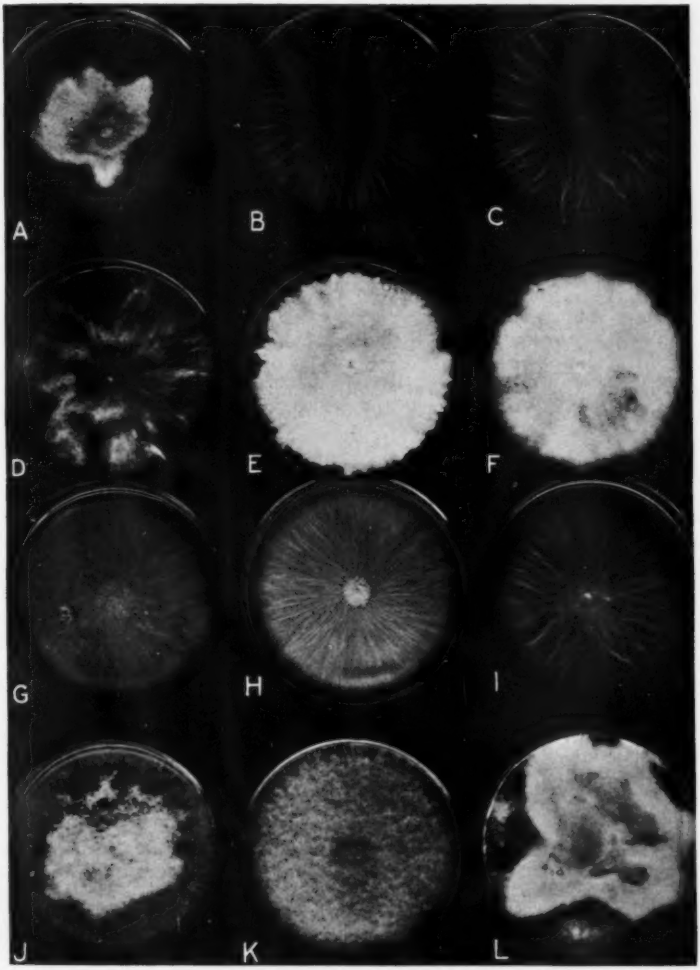

FIGURE 8.—Vascular *Fusaria* grown on a modification of Czapek's medium, containing sucrose as a source of carbon: A, 4b1; B, 2a2; C, 1a5; D, 7b5; E, 5b3; F, 4c4. All these are monoconidial cultures of *F. orthoceras* var. *pisi*. G, *F. conglutinans* var. *betae;* H, *F. conglutinans* var. *callistephi;* I, *F. conglutinans;* J, *F. niveum;* K, *F. tracheiphilum;* L, *Fusarium* sp. from celery. Note the range of colony character among the strains of *F. orthoceras* var. *pisi*, in comparison with the range of all the vascular *Fusaria* shown.

ment, regardless of the carbon source. The wilt strains acted as a unit where pronounced spiraling did occur, turning mostly in a clockwise direction except on galactose, where the direction was counterclockwise. Why this form of growth should have occurred is not clear.

No attempt was made to compare the fungi as to microscopic characters, but an examination of all plates was made to determine the kind and relative abundance of spore types. The wilt fungus is notably (*14*) a poor sporulator and as a rule little difference was found between strains. Very few macroconidia were observed on any of the media. Microconidia were produced sparingly by 5b and 7b, and to a less extent by 2a on sucrose, maltose, lactose, arabinose, and mannite. Chlamydospores were usually abundant, particularly on pectin. Those media poor in chlamydospore formation—xylose, galatose, and glucose—were also poor in all types of sporulation. Among the other fungi, *Fusarium conglutinans* var. *callistephi* and *F. tracheiphilum* were the most profuse sporulators, both as regards microconidia and macroconidia, exceeding all vascular *Fusaria* studied in that respect.

It appears that the vascular *Fusaria* utilize a wide range of carbohydrate sources, but do least well upon the pentoses, xylose and arabinose. This is in direct contrast to certain cortical *Fusaria* which have a preference for the pentoses (*6*). All seemed to do uniformly well upon pectin, especially after growth had proceeded far enough to counteract the high acidity of the medium. High concentrations of carbohydrate were used to accentuate color differences (*4*), and the data are therefore not exactly comparable to similar studies of this sort. However, the set-up given by Fahmy (*9*) in his work upon *F. vasinfectum*, was duplicated in another experiment, and comparable results were obtained. He found that the best growth of the cotton-wilt fungus occurred on glucose, galactose, sucrose, starch, and lactose in the order named.

DISCUSSION

Variability in monoconidial cultures of the *Fusaria* has been repeatedly observed. The genus *Fusarium* in fact contains some of the most outstanding instances of variation in colony character to be obtained through the use of single-spore methods (*12*). Brown (*5*) and recently Leonian (*13*) found, through the study of sectors from monoconidial parents, that cultural variability is accompanied by a different pathogenesis, usually in the form of a higher or lower virulence. In a survey of some 40 strains of *F. fructigenum* Fries, mostly saltants, Brown reported a wide range of cultural and virulence variability, and noted that the mycelial types were usually the more pathogenic. It was his conclusion that there should be a reduction in the number of species recognized on the basis that many specific names are applied to the variants of a species. Leonian compared the pathogenicities of a large collection of variants obtained from monoconidial cultures of *F. moniliforme* Sheldon. All degrees of virulence were encountered but there appeared to be no rule or order in the behavior of the dissociants, and it proved to be impossible to definitely correlate virulence with colony character.

All evidence from studies upon variation in fungi illustrate the hazard of using single-spore cultures in the study of a species exhibiting variation, unless large numbers of monoconidial cultures are employed. Otherwise only a single component of the species characters may be observed. Accordingly in these studies numbers of monoconidial lines were obtained for each strain, and from all of these types were chosen representatives of the species as obtained. In a portion of the pathogenicity studies the difficulty was obviated by using gross cultures.

The investigations reported here on *Fusarium orthoceras* var. *pisi* have yielded results somewhat comparable to those discussed above. Differences in colony character were at once apparent between original isolations of the fungus from different regions and localities. Differences of the same order between monoconidial lines were found. Moreover, within a given monoconidial line it was possible to assemble, through the phenomenon of dissociation, a group of cultures almost representative of the range in colony type and virulence exhibited by the entire group of strains. Thus a monoconidial parent has been shown in certain instances by its dissociants to possess the potentialities of most of the types of colony character and virulence of the 15 strains studied. It seemed that the careful selection of two or three parents would yield the entire range of strain differences. These variations, although wide, are considered within reasonable species limits. The variability in pathogenesis is always in respect to virulence upon susceptible varieties and bears no absolute correlation to the types of cultural variability. No resistant variety has been found susceptible to any culture, nor has any susceptible variety proven to be resistant to a fungal strain.

The occurrence of variability in the pea-wilt fungus again raises the question of strain origin. In addition to the variation within species, the very close interrelationship of the vascular *Fusaria* has suggested the operation of a sexual stage in nature resulting in the group of vascular parasites, or at least accounting for the variability in the specific groups, as segregants. Not to overlook the possibility that such a combination might be effected in artificial culture, the entire range of strains collected of the wilt fungus were planted side by side in different combinations on agar plates using different media. Other *Fusaria*, including strains of *Gibberella saubinetii* (Mont.) Sacc. and *G. moniliformis* (Sheldon) Wineland were also employed. These trials, although only superficial, gave negative results, but it still does not seem illogical that sexual fusions either occur now in nature producing a diversity of closely allied forms, or did occur, leaving a large assortment of segregants. So nearly, in fact, do some of the vascular Fusaria resemble one another, that it would seem that several evolved merely as variants or segregants in respect to pathogenicity. The Fusaria concerned in cabbage yellows, aster wilt, and beet wilt, for example, are readily distinguishable only by their parasitic properties, which, however, are sharply defined. These organisms have been termed respectively *F. conglutinans*, *F. conglutinans* var. *callistephi*, and *F. conglutinans* var. *betae*. The pea-wilt fungus, although maned *F. orthoceras* var. *pisi* includes within its range strains or variants which so overlap in colony character those of the above fungi as to be practically indistinguishable from them, except in pathogenesis which is again extremely clear cut. The only satisfactory means the pathologist has in differentiating between these four parasites (and possibly certain others) is upon the basis of parasitism, a quality which may logically be recorded in the varietal name of the fungus. In the same way other of the vascular *Fusaria* seem to be evolved from a group type with overlapping characters, and pathogenesis again effects the positive differentiation. There are obviously dangers attending to investing pathogenesis with specific ranking in mycologic nomenclature, but the pathogenic qualities are of great practical value in varietal delimitation. No

cross-inoculation trials between the vascular *Fusaria* and their various hosts have been successful. For the most part they are confined to one host species, and when fungal variations in pathogenicity have occurred, it involved varieties of the host species rather than new hosts.

Lacking knowledge of a sexual phase in the life histories of certain fungi, other explanations have been advanced to account for the variability displayed by the Fungi Imperfecti. Leonian (*13*) obviated the need of explaining the mechanism of variability by stating that dissociation is a phenomenon whereby a given organism traces the sphere of variability of the species, and it is therefore a highly natural and normal behavior. But even in this light a mechanism must operate in the appearance of the sudden change in a part of a colony where another phase of the species character comes into prominence. Brierley (*1, 2*) has proposed, while holding that the species in nature is of a collective kind, a mixochimaera theory in explanation of dissociation. Recently Hansen and Smith (*10*) have presented experimental data in support of this theory which they term "heterocaryosis," based upon nuclear assortment as a result of hyphal anastomosis. That anastomosis is of widespread occurrence among fungi is now an accepted fact, and also that not only unicellular spores but also hyphal cells may be multinucleate. The nuclei of these cells are not presumed to be genetically identical. Upon this basis Hansen and Smith postulate that a hyphal fusion gives rise to a new nuclear complex without nuclear fusion, and that the division of such cells yields units containing different nuclear assortments. It is the assortment of nuclei present in the cells of mycelium that they consider responsible for the thallus character, consequently where hyphal fusions occur in the fungal colony and subsequent cell division gives rise to a different nuclear assortment, a sector or variation originates. The occurrence of a multinucleate condition of unicellular spores accounts for the perpetuation in monoconidial lines of a diverse nuclear content. This theory of heterocaryosis provides a convenient explanation for most variation phenomena observed in the imperfect fungi where the spores contain two or more nuclei. The instances of variation in *F. orthoceras* var. *pisi*, with which this paper deals, might be explained under this theory. In those strains of relative stability, in regard to sectoring, such as 1a, the nuclei of the multinucleate hyphal cells may be considered largely of one kind. Unstable strains on the other hand may have a diverse assortment of nuclei. Thus variants might be expected to appear following anastomosis and nuclear reassortment (*10*), or as a result of spores being cut off bearing a change in the ratio of nuclear type or number (*7*), or following mycelial growth from a hyphal cell which did not get its proper quota of kind and number of nuclei. It would seem that convincing proof or disproof of the hypothesis might be obtained through an analytical study of the ascosporic cultures of those Gibberella species containing marked variation in the *Fusarium* stages. By this means thalli of a unicellular, uninucleate origin would be available for either study of the analysis or synthesis of dissociating colonies.

It appears, however, irrespective of the mode of strain origin, that there is a well-defined constancy in the kind of pathogenicity obtained with all strains of the pea-wilt *Fusarium*, and that the variability of the fungus is expressed in colony character and to some extent in virulence.

SUMMARY

Fifteen strains of *Fusarium orthoceras* var. *pisi*, the fungus causing pea wilt, were collected from eight States and compared in respect to pathogenicity and culture characters.

No evidence was found suggesting that different strains of the wilt fungus vary in host specialization.

Although all strains possessed the same kind of pathogenesis when tested upon a standard group of pea varieties, they varied somewhat in regard to the degree of pathogenesis.

All strains were arbitrarily grouped according to virulence. The differences in virulence were generally not extreme, but were encountered frequently both between monoconidial lines of a strain and between strains.

A comparison of monoconidial cultures representative of the strain types was marked by a wide range of variation in colony character. On the other hand comparison of the pea-wilt fungus with other vascular *Fusaria* showed a striking general similarity and overlapping of characters.

Type strains of the pea-wilt organism exhibited the same general temperature requirements upon artificial media and have a common optimum range for maximum radial growth upon agar plates.

Pronounced differences occurring on the same medium between different strains and between variants from the same single-spored parent thallus were recorded in respect to quality, intensity, rate of pigmentation of the medium, and other characters.

Fusarium orthoceras var. *pisi* and other vascular *Fusaria* studied, showed a wide carbon source preference, growing well on all media tried except the pentoses, xylose and arabinose. Strain differences were brought out to some extent upon these media.

Variation as it occurs in *Fusarium orthoceras* var. *pisi* seems to fluctuate within type limits defined on the one hand by certain general culture characteristics and on the other hand by a rather specific behavior in respect to pathogenicity.

No strain encountered was considered sufficiently divergent in type from the rest to justify the creation of new nomenclature. Indeed, it would rather seem advantageous to simplify the existing nomenclature of the vascular *Fusaria* by a condensation under one species name of those parasites, the culture characters of which so overlap as to make ordinary mycological methods inadequate in their differentiation, distinguishing such parasites by varietal names based upon host pathogenesis.

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INHERITANCE OF REACTION OF WHEAT TO PHYSIOLOGIC FORMS OF TILLETIA LEVIS AND T. TRITICI [1]

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INTRODUCTION

Recent studies throughout the main wheat-growing regions of the world have demonstrated the existence of numerous physiologic forms within *Tilletia levis* Kuehn and *T. tritici* (Bjerk.) Wint., the two species of fungi causing bunt in wheat.[3] The presence of these forms makes the study of inheritance of resistance in wheat crosses somewhat more complicated than previously.

When the situation is considered from the economic point of view, it is apparent that in regions in which numerous forms are present the breeding of varieties resistant to bunt and desirable in other respects can now proceed with more assurance of success. Consideration, however, should be given to the fact that hybridization between physiologic forms and the possible production of new forms have been demonstrated (*11*).[4] In the Pacific Northwest only one form of *Tilletia tritici* was known to be present prior to 1918. Recent surveys (*18*) have shown that physiologic forms of *T. levis* and new forms of *T. tritici* are now widespread throughout the wheat-growing portions of this area. Heald and George (*17*) have shown that seed treatment with various fungicides, effective elsewhere, is of limited value for winter wheat in this area. Moreover, some varieties that are resistant when inoculated with the smut prevalent a decade ago are very susceptible to some of the recent forms. A promising method of coping with this condition lies in the production of new varieties resistant to these fungi and possessing other desirable qualities. Progress can be made more rapidly in this undertaking when the mode of inheritance of reaction to bunt in various varietal crosses is known.

PREVIOUS WORK

Earlier studies on the genetics of reaction to bunt have been reviewed by Briggs (*2*) and need not be considered here in detail; only the more pertinent features will be mentioned.

In all the investigations reported, the rough-spored species, *Tilletia tritici*, has been used as the inoculum. Gaines (*12, 13*) and Gaines

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[2] The writer is indebted to Dr. F. D. Heald, head of the Department of Plant Pathology, State College of Washington, for suggestions regarding the manuscript; to Dr. E. F. Gaines, professor of genetics, State College of Washington, for suggestions and criticisms in the course of the study; and to members of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, for suggestions arising from a critical reading of the manuscript.

[3] A third species of Tilletia infecting wheat was discovered recently in India (*21*).

[4] Reference is made by number (italic) to Literature Cited, p. 104.

and Singleton (*14*) found that in the inheritance of reaction to bunt in the crosses studied many factors were concerned, the number of which could not be determined except in a cross between Marquis and Turkey in which a 2-factor difference was indicated. In some crosses, resistance was inherited as a recessive. In two crosses, well-defined transgressive segregation occurred, some of the progenies being more resistant and others more susceptible than the parents.

Briggs (*2*) studied the inheritance of reaction to bunt in hybrids between three susceptible wheats and two wheats (Martin and Hussar) which are resistant to the physiologic form of bunt used in the investigations at the California Agricultural Experiment Station. It was shown that resistance behaves as a dominant in inheritance in these crosses. A single main factor was found to govern the resistance of Martin, whereas Hussar was found to differ from the susceptible wheats by at least two dominant factors, one of which is the same as the factor in Martin. The expression of a second factor is described in a recent publication (*4*). White Odessa, another variety resistant to the form of bunt used in the experiments of Briggs, has been found (*5*) to differ from susceptible wheats by one main dominant factor. Briggs (*6*) has also shown that Banner Berkeley carries the same factor for resistance as Martin.

In Germany a number of crosses between spring and winter wheats were investigated for reaction to bunt by Knorr (*19*). His results indicated that resistance was inherited as a recessive; the number of factors involved could not be determined. In crosses between winter wheats, Giesecke (*15*) concluded that resistance was recessive and that multiple factors were responsible for the results obtained. Transgressive segregation was found in a cross between two moderately susceptible wheats.

Because the presence of physiologic forms in the two species of fungi causing bunt was discovered only recently, no studies on the reaction of progenies of a cross to different forms have as yet been described; nor have investigations been reported on the inheritance of reaction to *Tilletia levis*.[5] Consideration was given to these phases in the investigation reported herein.

MATERIALS

Hope (*Triticum vulgare*[6] Vill.) (C. I. 8178)[7], a spring wheat, was one of the parents in all crosses tested in this study. It is described by Clark and Ausemus (*7*), and McFadden (*20*) discusses its agronomic qualities. Of the other parents, Jenkin (*T. compactum* Host) (C. I. 5177) was the only spring wheat; it is described by Clark, Martin, and Ball (*9*). White Odessa (C. I. 4651) and Ridit (C. I. 6703), varieties of *T. vulgare*, were the winter-wheat parents; both are described by Tisdale et al. (*24*), and additional data on Ridit are presented by Clark, Love, and Parker (*8*).

Three so-called physiologic forms of *Tilletia tritici* (T–1, T–2, T–3) and two forms of *T. levis* (L–4, L–5) were used in the tests. These forms were isolated from collections of bunt made in various parts of

[5] Since this manuscript was completed, the reactions of two crosses to two collections of *Tilletia levis* and two of *T. tritici* have been described (*1*).

[6] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum*, but as *T. vulgare* is in general use among agronomists, the writer gives preference to that form.

[7] Accession number of the Division of Cereal Crops and Diseases.

the Pacific Northwest and were obtained by culturing on differential hosts. The reaction of some of the parental varieties to these forms can be shown in the results of a rod-row test made in 1929, the results of which are in close agreement with those of a similar test made in 1930. In both seasons, environmental conditions were very favorable for infection, as evidenced by high percentages of bunt on the susceptible varieties. The results of the test made in 1929 are presented in table 1.

TABLE 1.—*Reaction of varieties of wheat to three forms of Tilletia tritici and two forms of T. levis, 1929*

Variety	C. I. No.	Percentage of wheat attacked by indicated form of bunt				
		T. tritici			T. levis	
		T-1	T-2	T-3	L-4	L-5
Hybrid 128	4512	68	83	90	84	90
Turkey	6175	6	86	2	71	72
Albit	8275	0	75	77	44	0
Martin	4463	0	19	71	51	0
Hussar	4843	0	25	0	39	0
Ridit	6703	0	2	0	6	3
Hope (fall sown)	8178	51	60	42	53	78
Hope (spring sown)	8178	0	0	0	0	0
Jenkin (spring sown)	5177	92	78	73	58	56

In table 1 the five physiologic forms of Tilletia are clearly differentiated on the two winter wheats, Martin and Hussar. T–2 caused 25 percent of bunt on Hussar and 19 percent on Martin, whereas these wheats were smut free when inoculated with T–1. Martin distinguishes T–1 from T–3. The 25 percent on Hussar with T–2 is in sharp contrast to zero with T–3.

Martin, however, is equally effective as a differential between T–2 and T–3. Bunt developing on Martin (*23*) after inoculation with T–2 is very different from that caused by any other form of the fungus under test. T–2 gives rise to smut balls which at maturity are only slightly larger than normal ovaries before fertilization; of the spores which they contain, some appear mature, but a large proportion have unthickened or partly thickened walls and seem to be in various stages of development. Because of the partly developed smut balls, heads of Martin infected with T–2 resemble sterile heads in external appearance, and can readily be distinguished from heads of this variety infected with any of the other four physiologic forms. Albit, Martin, and Hussar serve to differentiate the two forms of *Tilletia levis*.

A peculiar condition has been observed in Hope. Prior to the growing season of 1929, Hope had been highly resistant to bunt in tests at Pullman, Wash. The winter of 1928–29 was mild, and seeds of this variety inoculated with different collections of bunt and sown in the fall of 1928 produced plants which lived through the winter and exhibited at maturity from 42 to 78 percent of bunt. Later trials have confirmed the conclusion that Hope, when sown at the normal seeding time in spring, is resistant to all forms of bunt with which it has been tested, but when sown in the fall is susceptible. This condition is described in greater detail in another paper (*22*)

METHODS

The parental material and hybrids were grown in the nursery field which forms a part of the farm of the State College of Washington. The field is divided into three parts, and on these a 3-year rotation (sweetclover, summer-fallow, cereal nursery) is maintained. The summer-fallow is kept free from plant growth, and thus the possibility of the appearance of volunteer grain in the experiments is reduced to a minimum. F_1 and F_2 hybrids were protected as much as possible from bunt infection by treatment with copper carbonate.

The necessity of maintaining the physiologic forms of the fungi free from contamination is obvious. Judicious selection of a host variety for each form has been of much service. For example, from the results presented in table 1, if inoculum of T–2 were collected on Hussar neither T–1 nor T–3 would be present. Similarly, if T–3 were collected on Martin, neither T–1 nor T–2 would be present, provided the abnormal smut heads from an infection of this variety by T–2 were not overlooked. It is recognized that the five forms mentioned above may not be pure; some of them may be composites of two or more forms or may be heterozygous for pathogenicity. However, in testing these forms on various hosts and using inoculum from different varieties in two growing seasons, there has been no indication of mixture.

Inoculum of each physiologic form was obtained from selected varieties as soon as the crop had matured. Hybrid seeds were counted out and arranged for inoculation along with the necessary checks. Inoculation was made by placing a quantity of powdered smut in a glass vial with each lot of seeds and shaking vigorously until the seeds appeared well blackened. The excess smut not adhering to the seeds was then sifted out. During these operations every precaution was taken to prevent the contamination of any form by spores from any other.

Sowing was done in either rod rows or ½-rod rows, the seeds being sown approximately 2½ inches apart to facilitate the separation of single plants at harvest. Care was taken that all rows and different parts of each row should be, as far as possible, of the same depth, approximately 3 inches. The seed for each experiment was sown on the same day, except in one instance when sowing extended over two consecutive days.

It is recognized that in certain parts of the Pacific Northwest at the time of fall seeding the surface soil may be heavily contaminated with wind-blown spores of Tilletia. By means of spore traps, Heald and George (*17*) in 1916 demonstrated a fall of over 13,000 spores per square inch in a period of seven days when the dissemination of spores was at its height. Sterilization of the surface soil in the field is not practicable and may be undesirable since it may upset the biological balance in the soil. In spite of this possible source of contamination, no difficulty has been experienced in keeping the forms of bunt pure; and there has been ample opportunity in these tests for admixtures to become apparent. For example, considering some of the varieties listed in table 1, in the series inoculated with T–1, any admixture of T–2, T–3, or L–4 would smut Albit and Martin; likewise, admixture of T–2 or L–4 would smut Hussar. Small percentages of bunt would also be apparent on certain varieties in some of the other four series and in other wheats not listed in table 1. No such infections have been found in tests conducted over a period of two years. The absence of contamination may be accounted for

by the fact that (1) wind-blown spores were almost entirely of T–1, which does not smut the differential wheats Albit, Martin, and Hussar, and (2) all seeds, when planted, carried the maximum spore load, which may be in the neighborhood of 150,000 spores per kernel (*16*).

At harvest the data on bunt were recorded by grouping the plants according to the proportion of smutty heads. In studies on the inheritance of reaction to bunt, different methods of determining the reaction of parents and progenies have been employed. Gaines (*13*) separated the plants of each row into three piles—bunt free, wholly bunted, and partly bunted—and recorded the number of plants in each. The total percentage of bunt in each row was computed by the formula $a \times b + c = d$, in which, in any row:

a = percentage of bunted heads in the partly bunted plants,
b = percentage of partly bunted plants,
c = percentage of wholly bunted plants, and
d = total percentage of bunt.

Briggs (*2*) maintained that in a study of inheritance the plant should be considered as the unit. He separated the plants in each row into two piles, bunt free and bunted. The total number of plants and the number of bunted plants were recorded and the percentage of bunt calculated. A plant was classed as bunted if it showed a trace of bunt. Both Giesecke (*15*) and Knorr (*19*) have employed this method in inheritance studies.

In comparing the bunt reaction of different varieties of wheat it seems that, while two varieties may have the same proportion of plants bunt free, one variety may have consistently a large proportion of plants with a trace of bunt, while the other may have a large proportion of the plants wholly bunted. If, under such conditions, all plants showing any degree of smutting are placed in one group, genetic differences in reaction may be concealed. This point is illustrated in table 2.

TABLE 2.—*Distribution of plants among the five bunt-percentage groups and percentage of bunt in each row of duplicate rows of Hybrid 128 and Martin wheat inoculated with physiologic form T–2 and grown in 1930*

Variety	Row No.	Number of plants in bunt-percentage group indicated					Total number of plants	Percentage of bunt in row
		0	20	50	80	100		
Hybrid 128	1	5	0	1	2	20	28	79
	291	3	1	0	3	26	33	87
Martin	5	3	7	5	6	0	21	41
	299	4	12	8	4	1	29	* 37

* Percentage of bunt in row 299 $= \frac{(20\times12)+(50\times8)+(80\times4)+(100\times1)}{29} = 37$

In the present study the plants in a row were first pulled, then sorted into five bunt-percentage groups as follows:

0 percent = all heads bunt-free.
20 percent = up to one-third of heads bunted.
50 percent = one-third to two-thirds of heads bunted.
80 percent = above two-thirds, but not all, of heads bunted.
100 percent = all heads bunted.

A spike which contained both kernels and smut balls was considered smutty. The number of plants in each of the five groups was then

counted and recorded. Computation of the percentage of bunt is illustrated in row 299 of table 2; calculation was made to the first decimal place in percentages less than 10 and to the nearest whole number in those greater than 10.

It is apparent from table 2 that the number of bunt-free plants in the two varieties, Hybrid 128 and Martin, is approximately the same, whereas the distribution among the bunt-percentage groups is strikingly different. The results with these varieties in 1929 corresponded closely to those obtained in 1930. It is apparent that if, in this example, percentage of bunt is based on two groups, bunt-free and bunted, the percentage of bunt in the two varieties is approximately the same; with reference to the degree of smutting, however (table 2), the percentage of bunt in one variety is approximately double that in the other. Differences of a similar nature, though usually not so marked, have been observed in other varieties. It would be conceded that these differences between varieties are due to differences in reaction. Similar differences would be expected between hybrids. It may be advantageous, therefore, in the genetical study of reaction to smut, to record the data in such a manner as to show the plant distribution with respect to degree of smutting in each row as well as the total percentage of smut.

EXPERIMENTAL RESULTS

REACTION OF HOPE × JENKIN TO PHYSIOLOGIC FORMS OF BUNT

Crosses were made between Hope and Jenkin in 1927, and the first generation was grown in 1928. In the spring of 1929 the seeds obtained from the F_1 plants were spaced 4 to 5 inches apart in rows 18 inches apart to allow for the vigorous development of each plant; each F_2 plant consequently gave a good yield of seed. Hope, when sown in spring, has been resistant to all the physiologic forms of bunt used in the tests at Pullman, whereas Jenkin has been susceptible to all forms. One of the objects of the present study was to determine whether the factors responsible for resistance to any one form were the same as those for resistance to any other form. Other phases under investigation were (1) the number of factors concerned in the inheritance of reaction to various bunt forms in this cross, and (2) the relationship, if any, between reaction to bunt and such characters as glume color and length of awn. Accordingly, from each of 195 F_2 plants, three sets of 75 seeds were counted out. In each of the three series a pair of check rows, one of each parent, was included after every tenth hybrid row. The first series was inoculated with T–3, the second with L–5, and the third with a mixture of T–1, T–2, and L–4. Data were recorded in the manner described, and the distribution of progenies and parents, according to their reaction to the forms of Tilletia, is shown in table 3 and in figure 1.

In the study of the reaction of wheat varieties and hybrids to bunt it has been observed (*14, p. 170*) that a given percentage difference between two resistant types is much more significant than the same percentage difference between two susceptible types; that, for example, a segregate or variety

which produces an average of 1 percent of bunt is evidently quite different from a variety which produces 10 percent, but a variety which produces 70 percent is not so different from one which produces 80 percent.

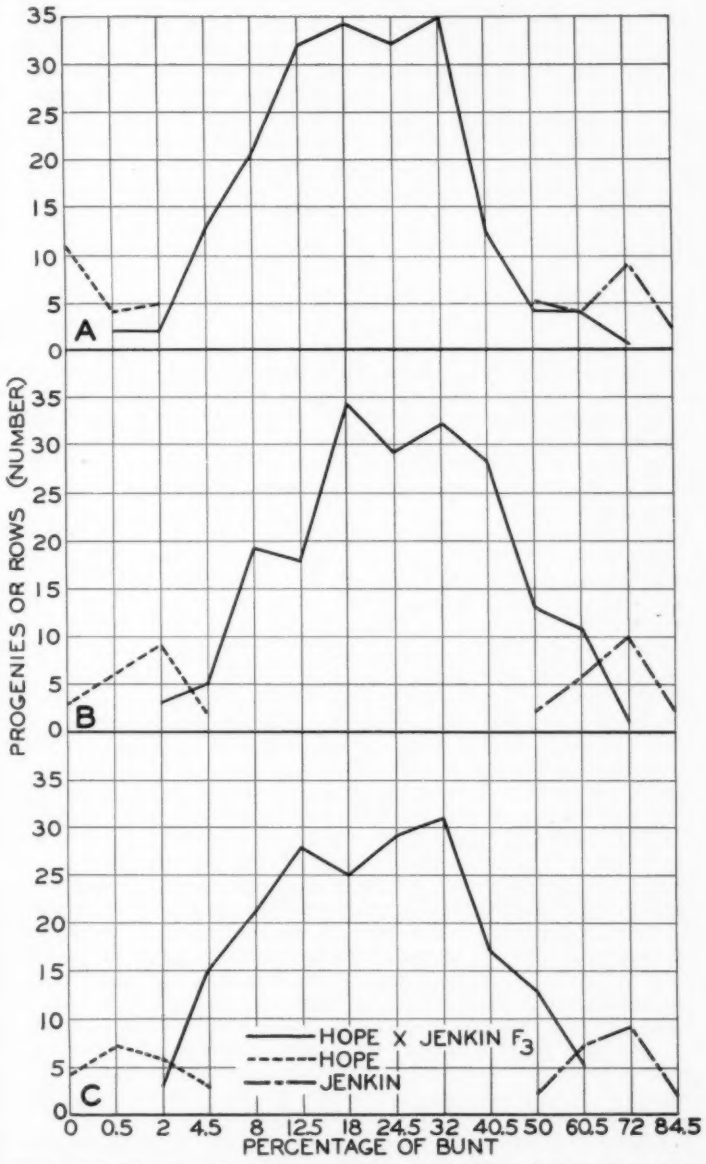

FIGURE 1.—Distribution among bunt-percentage classes of parental rows and F_3 progenies of Hope × Jenkin in three series inoculated with different physiologic forms and sown April 6, 1930: A, Series 1, inoculated with T–3; B, series 2, inoculated with L–5; C, series 3, inoculated with a mixture of T–1, T–2, and L–4.

TABLE 3.—*Percentage of bunt on parents and F_3 progenies of Hope × Jenkin when inoculated with different physiologic forms and sown on April 6, 1930*

Parent or F_3	Series No.	Inoculated with form—	Number of rows in the bunt-percentage class indicated														Total number of rows
			0	0.5	2	4.5	8	12.5	18	24.5	32	40.5	50	60.5	72	84.5	
Hope	1	T-3	11	4	5	---	---	---	---	---	---	---	---	---	---	---	20
Jenkin			---	---	---	---	---	---	---	---	---	---	5	4	9	2	20
Hope × Jenkin			---	---	2	2	13	21	32	34	32	35	12	4	4	1	192
Hope	2	L-5	3	6	9	2	---	---	---	---	---	---	---	---	---	---	20
Jenkin			---	---	---	---	---	---	---	---	---	---	2	6	10	2	20
Hope × Jenkin			---	---	---	3	5	19	18	34	29	32	28	13	11	1	193
Hope	3	T-1, T-2, and L-4 (mixture)	4	7	6	3	---	---	---	---	---	---	---	---	---	---	20
Jenkin			---	---	---	---	---	---	---	---	---	---	2	7	9	2	20
Hope × Jenkin			---	---	3	15	21	28	25	29	31	17	13	5	---	---	187

In the classification of Gaines and Singleton the differences between the class centers, with the exception of the difference between the first two classes, constitute an arithmetical progression in which the values increase by 2.5. Other investigators of the inheritance of reaction of cereals to the various smuts have grouped hybrid and parental rows in classes of 5 or 10 percent of smut. It may be desirable, however, in a study of the distribution of hybrid progenies and parents in reaction to smut, to arrange the progenies and parental rows in classes which are of approximately equal value in resistance or susceptibility.

In the present study (table 3), the first class contains rows showing no smut, while for the other classes the class intervals form an arithmetical progression in which the interval of the first is one and that of the others increases by one. Such a classification gives some 14 or 15 classes; if the number of classes is less than these, tendencies in distribution may not become apparent. Perhaps the best test of the validity of any such method is an examination of the distribution of the check rows. Since the parental rows were distributed in pairs—one row of Hope and one of Jenkin—throughout the nursery, the resistant parent was subjected to approximately the same environmental conditions and should show the same fluctuations as the susceptible parent. The data in table 3 show that Jenkin fluctuated among four classes in each of the three series. A similar situation was found in Hope. The reactions ranged through 4 classes in series 2 and 3 and probably would have ranged through 4 in series 1 if the degrees of resistance in the 11 rows showing no bunt had been given. Moreover, examination of the reaction of parental rows in table 7 indicates that the distribution through reaction classes of types intermediate between highly susceptible and highly resistant, e.g., White Odessa, is similar to that found in the extremes as represented by Hope and Jenkin in table 3.

Figure 1 shows clearly the high resistance of Hope and the high susceptibility of Jenkin in each of the three series. Because of the sharply contrasting reactions of the two parents, the nature of the segregation of bunt reaction in the F_3 progenies can be more readily determined.

Dominance of either resistance or susceptibility has frequently been ascertained from a comparison of the reaction of the F_1 hybrids with the parents. However, in a segregation involving a number of factor pairs, the expression in the F_1 plants may be the result of the dominance of resistance in certain factor pairs and the dominance of sus-

ceptibility in others. Only by a study of each factor pair can dominance be accurately determined. This has not been attempted in the Hope × Jenkin cross. However, a comparison of the reaction of the F_3 progenies with that of the parents (fig. 1) gives no indication of the dominance of either resistance or susceptibility.

There is a striking resemblance between the distribution of progenies in each of the three series, a condition which suggests the interaction of the same number of factors in each series. Before investigating the possible number of factors involved, the three series may be compared in more detail.

It has been mentioned that, of each hybrid progeny, one part was inoculated with T–3, a second part with L–5, and a third with a mixture of T–1, T–2, and L–4. The percentages of bunt in these portions of each progeny have been correlated, and the results are shown in table 4.

TABLE 4.—*Correlations between series containing portions of F_3 progenies of Hope × Jenkin, inoculated with different physiologic forms of bunt, series 1 with T–3, series 2 with L–5, and series 3 with a mixture of T–1, T–2, and L–4*

Series correlated	Number of progenies in the comparison	Coefficient of correlation
1 with 2	190	0.69±0.03
1 with 3	184	.69± .03
2 with 3	186	.66± .03

The data in table 4 show that in each of the three comparisons there is a significant and high degree of correlation between the percentage of bunt produced in the several portions of each progeny. The value and similarity of the coefficients of correlation indicate that at least some of the factors responsible for resistance to one form are the same as those responsible for resistance to the other forms.

The relationship between the series may be determined more exactly by examination of the correlation tables. Since the three tables are similar, only one will be presented. The correlation between series 1 and series 3 appears as table 5.

TABLE 5.—*Correlation between the percentages of bunt in two portions of each of 184 F_3 progenies of Hope × Jenkin, one portion inoculated with physiologic form T–3 (series 1), the other with a mixture of physiologic forms T–1, T–2, and L–4 (series 3)*

Bunt-percentage class in series 1	Number of plants in indicated bunt-percentage class in series 3										Total
	2	4.5	8	12.5	18	24.5	32	40.5	50	60.5	
0.5	1		1								2
2			1	1							2
4.5	1	5	3	3			1				13
8		4	9	3	2	2					20
12.5	1	3	2	8	6	2	5	1			28
18		1	3	9	5	5	6	2	2		33
24.5			1	1	4	10	8	5	1		30
32			1	3	6	6	9	6	3	1	35
40.5					2	2	2	2	3	1	12
50						2			2		4
60.5								1	1	2	4
72										1	1
Total	3	13	21	28	25	29	31	17	12	5	184

In the distribution of data in table 5 for the 12.5 percent class of series 1, the array of series 3 extends through 8 classes; similarly, in the 18 percent class and 32 percent class of series 1, the array of series 3 is distributed through 8 classes, whereas the arrays of the other series extend through only 7 classes or less. The percentage of bunt in the Jenkin check rows of each of the three series (table 3) fluctuates among 4 classes; a similar variation exists in the Hope check rows. If, therefore, the percentages of bunt in the Jenkin check rows of series 1, for example, were correlated with corresponding Jenkin rows in series 3, in no case would one class of one series have an array of more than four classes of the other series. All rows of Jenkin, however, have the same genotypic constitution. The condition in the hybrids is somewhat different. In any class of each series there is a variety of genotypes. Assuming that the fluctuations in percentage of bunt in the hybrids are the same as those in the parents, the genotypes in any one class of the hybrids could theoretically give fluctuations among three classes on either side of that class, giving in all a range of seven classes. In table 5 variations through eight classes, instead of seven, occur, but this difference is probably not significant. If, therefore, a correlation table were made from two series that had been inoculated with the same physiologic form of bunt instead of with different forms as in table 5, the expected distribution, on the basis of the fluctuations found in the parental rows, would not differ materially from that presented in table 5. It may therefore be concluded that the factors in Hope responsible for resistance to the bunt form used as inoculum in series 1 (T–3) are the same as those responsible for resistance to the forms used in series 3 (T–1, T–2, and L–4). Since a similar situation is found in the correlation tables of series 1 with 2 and series 2 with 3, the conclusion may be extended to include resistance to the form of smut with which series 2 (L–5) was inoculated. The similarity of the distribution of hybrid rows in the three series in figure 1 adds weight to this conclusion.

One striking feature in a factorial analysis of the data (table 3) is the relatively small number of resistant progenies and the entire absence of bunt-free progenies; of equal significance is the small number of progenies as susceptible as the Jenkin parent. These features, together with the great preponderance of progenies having reactions intermediate between those of the two parents, indicate the presence of a number of factors. Because of the importance of extremes of susceptibility and resistance in the genetic analysis of the data, the plant distribution in some of the most resistant progeny rows, together with that of the nearest check rows of Hope, is given in table 6.

Evidence has been presented which indicates that in this cross the factors for resistance against the invasion of the plant by any one of the physiologic forms of bunt tested are the same as those for resistance against any other form used in this study. Therefore, any progeny that has the same genotype as Hope should not have a higher percentage of bunt in any of the three series than would be possible in Hope under the conditions of this experiment. The probable error in terms of percentage of bunt can not be used to advantage in determining the potential range of the parents under these conditions because of the fact that in a study of this nature a given range in percentage of bunt, e.g., 5 percent, is of smaller significance at the sus-

ceptible end of the curve than at the resistant end. Approximate limits, however, may be defined from a study of the distribution of the parental rows through the bunt-reaction classes. Such a determination may be made from the data on the parents in table 3. Moreover, it may be added that the highest percentage of bunt in Hope in series 1 is 2.4; in series 2, 3.0; and in series 3, 4.2. From table 6 it appears that on this basis only progenies 860–35 and 861–16 come within the probable limits of Hope in all three series. However, Briggs (*3*) has shown that modifying factors may operate in the reaction to bunt of certain crosses. Allowing for the operation of such factors in addition to the main factors in the Hope × Jenkin hybrids, progenies 861–62 and 861–66 might also be considered to have the same main factors as Hope, giving in all from a total of 195 progenies 4 as resistant as the resistant parent. The other 3 progenies listed in table 6 show significantly large deviations from the reaction of Hope.

TABLE 6.—*Distribution in bunt-percentage groups of plants in the seven most resistant F_3 progenies of Hope × Jenkin compared with that in the nearest check rows of Hope and with that of all check rows of Hope and Jenkin, in each of the three series*

Progeny no. or variety	Series No.	Number of plants in the bunt-percentage group indicated					Percentage of bunt in row
		0	20	50	80	100	
860-35	1	46	2	0	0	0	0.8
Hope	1	36	0	0	0	0	0
860-35	2	42	2	1	0	0	2.0
Hope	2	44	5	1	0	0	3.0
860-35	3	39	4	0	0	0	1.9
Hope	3	30	3	1	0	0	3.2
861-16	1	42	6	1	0	0	3.5
Hope	1	28	1	0	0	0	.7
861-16	2	45	9	0	0	0	3.3
Hope	2	43	1	0	0	0	.5
861-16	3	47	2	2	0	0	2.7
Hope	3	39	3	0	0	0	1.4
861-62	1	52	4	2	0	0	3.1
Hope	1	35	2	1	0	0	2.4
861-62	2	39	15	0	1	0	6.9
Hope	2	37	1	0	0	0	.5
861-62	3	38	10	2	0	0	6.0
Hope	3	39	5	0	0	0	2.3
861-66	1	41	4	0	0	0	1.8
Hope	1	35	0	1	0	0	1.4
861-66	2	50	5	1	0	0	2.7
Hope	2	46	4	0	0	0	1.6
861-66	3	30	17	0	0	0	7.2
Hope	3	43	7	0	0	0	2.8
861-69	1	40	4	1	0	0	2.9
Hope	1	35	0	1	0	0	1.4
861-69	2	26	15	3	2	1	15.1
Hope	2	46	4	0	0	0	1.6
861-69	3	27	9	3	2	0	12.0
Hope	3	43	7	0	0	0	2.8
861-81	1	31	9	2	1	0	8.4
Hope	1	44	0	0	0	0	0
861-81	2	31	5	0	0	0	2.8
Hope	2	39	2	0	0	0	1.0
861-81	3	43	3	2	1	0	4.9
Hope	3	34	9	0	0	0	4.2
861-109	1	48	2	0	0	0	.8
Hope	1	42	0	0	0	0	0
861-109	2	48	10	0	0	0	3.4
Hope	2	31	2	0	0	0	1.2
861-109	3	41	10	3	3	0	10.4
Hope	3	38	5	0	0	0	2.3
Hope (all checks)	1	725	9	4	0	0	.5
	2	791	41	5	1	0	1.4
	3	825	52	2	1	0	1.4
Jenkin (all checks)	1	193	54	85	229	333	63.7
	2	183	48	68	200	425	68.0
	3	210	61	51	189	427	65.7

In a similar manner a comparison was made between the reactions of the most susceptible progenies and those of the susceptible parent. Consideration was given, as in the resistant progenies, to the percentage of bunt in each row, but of much significance is the distribution of the plants of each row in the five bunt-percentage groups. The typical distribution for Jenkin in the three series is given in table 6. Allowing for the presence of modifying factors, only four progenies were considered to have the same main factors for reaction to bunt as Jenkin.

In view of these conclusions concerning the 4 resistant and 4 susceptible progenies and the general distribution of the 195 progenies with respect to reaction to bunt in the three series, it is probable that under the conditions of this experiment Hope and Jenkin differ by three main factors in reaction to the five physiologic forms of bunt.

REACTION OF HOPE × WHITE ODESSA AND OF HOPE × RIDIT TO FORM L–4

Crosses were made in 1926 between Hope and each of the two winter wheats, White Odessa and Ridit. F_1 plants were grown in 1927. F_2 plants were grown in 1928 from seeds treated with copper carbonate to reduce bunt infection to a minimum. From the seed produced by each F_2 plant two sets of 38 seeds each were counted out. One series was sown in the fall of 1928 and the other in the spring of 1929, each lot occupying a half-rod row; both series were inoculated with L–4. After every 20 rows of hybrids a row of each parent was sown. In the spring-sown material scarcely a trace of bunt appeared, although susceptible wheats inoculated with the same physiologic form and sown at the same time showed up to 56 percent of bunt. Apparently the winter wheats carry at least some of the same factors as Hope for resistance to L–4 in spring seeding. The data on the amount of bunt in these crosses in the fall-sown series are presented in table 7 and are shown graphically in figure 2.

TABLE 7.—*Distribution in bunt-percentage classes of parents and F_3 progenies of Hope × White Odessa and Hope × Ridit, inoculated with physiologic form L–4 and sown October 13, 1928*

Parent or F_3	Number of rows in bunt-percentage class indicated													Total number of rows
	0	0.5	2	4.5	8	12.5	18	24.5	32	40.5	50	60.5	72	
Hope	----	----	----	----	----	----	1	2	1	1	2	2	----	9
White Odessa	----	----	----	2	4	4	1	1	----	----	----	----	----	12
Hope × White Odessa	1	----	2	4	10	17	21	48	32	32	34	18	5	224
Hope	----	----	----	----	----	----	----	2	5	----	1	2	----	10
Ridit	6	2	3	1	----	----	----	----	----	----	----	----	----	12
Hope × Ridit	37	15	20	8	28	21	14	22	18	12	14	4	2	215

In summarizing the reactions of the progenies of the two crosses in table 7, only progenies with 15 or more plants were included. It would have been desirable to eliminate also parental rows having less than 15 plants. However, winterkilling and poor germination considerably reduced the number of plants in the rows of the Hope parent; rows having 11 or more plants were therefore included in table 7. Even on this basis the number of rows of Hope is less than that of the other parent in both crosses. All rows of White Odessa and Ridit contained more than 15 plants.

It will be noted in table 7 that the reactions of the parent varieties to form L–4 fluctuate among 4 to 6 classes, whereas in table 3 the parental reactions range through only 4 classes. However, as was pointed out previously, the Hope × White Odessa and Hope × Ridit

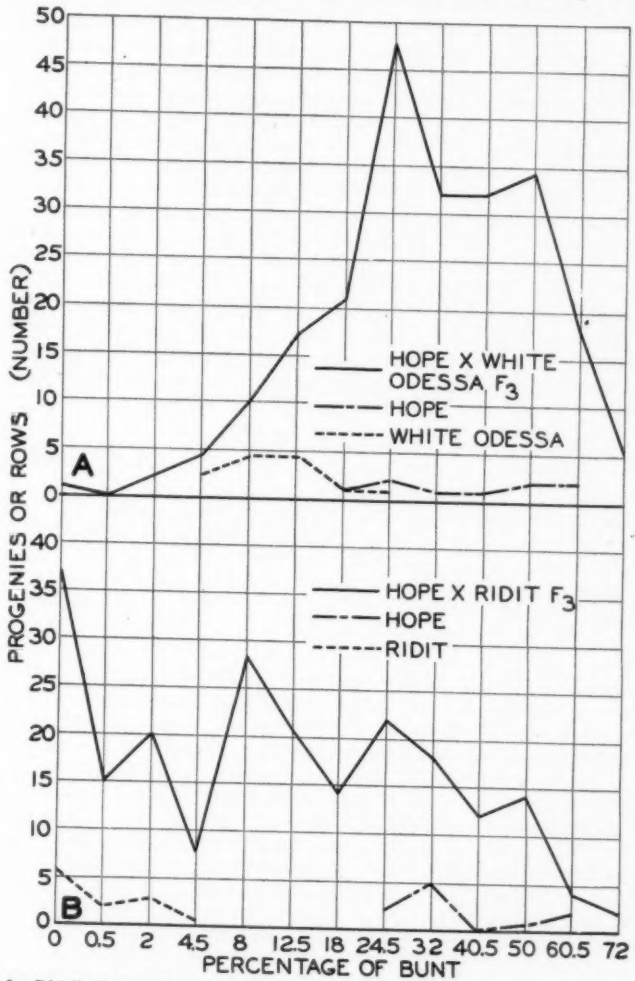

FIGURE 2.—Distribution among bunt-percentage classes of parental rows and F_3 progenies of (A) Hope × White Odessa and (B) Hope × Ridit inoculated with physiologic form L–4 and sown October 13, 1928.

populations were tested in ½-rod rows while the Hope × Jenkin cross was tested in rod rows. It is apparent that the rod-row test is much superior to that made in the ½-rod row for the analysis of the genetic factors for reaction to bunt.

The number of factors concerned in the Hope × White Odessa progenies can not be determined from this test, because the reactions of the parental rows overlap and show considerable variability owing to the small number of plants per row. One progeny consisting of 32 plants was free from bunt. Seeds from the same F_2 plant were inoculated with L–4 and sown along with the parents and other progenies in the fall of 1929. Although this progeny was not immune in the retest, it was more resistant than the White Odessa parent. There is a strong indication that much natural crossing occurs at Pullman in certain seasons, and it is possible that this progeny is the result of a natural cross on one of the F_1 plants. It may also be the result of an admixture. Such an interpretation is strengthened by the fact that the general distribution of the progenies (fig. 2, A) does not indicate that the reaction of this progeny is the result of transgressive segregation.

In the Hope × Ridit cross a different situation exists. The high resistance of Ridit is sharply contrasted with the moderate susceptibility of Hope. A single-factor difference for reaction to this form is suggested from the distribution of the progenies. A more accurate interpretation, however, can be made from examination of the degree of smutting of plants within each progeny and each parental row. The reactions of the parents planted at intervals among the hybrid progenies may be compared in the summary presented as table 8.

TABLE 8.—*Percentage of plants in the five bunt-percentage groups in the parental rows as distributed among the Hope × White Odessa and Hope × Ridit F_3 populations*

Variety	Percentage of plants in bunt-percentage group indicated					Number of—	
	0	20	50	80	100	Plants	Rows
Hope	45	8	14	22	11	279	19
White Odessa	73	14	10	2	1	346	12
Ridit	97	3	.6	0	0	327	12

The data of table 8 show that of 327 plants in the Ridit rows only 3 per cent were in the 20 per cent bunt group and only 0.6 per cent in the 50 per cent group. Progenies having the resistance of Ridit therefore would not have any wholly bunted plants. Furthermore, 29 F_3 progenies of Hope × Ridit, the reaction of which could be classified with difficulty, were retested in 1930 with the same form of bunt. Using these data in connection with the percentages of bunt in the rows of the progenies and the parents, the hybrids were classified, and the resulting data were tested for goodness of fit (*10*) to a 1:2:1 ratio in table 9. A value of 0.16 for P indicates a reasonably good fit.

TABLE 9.—*Test for goodness of fit to a 1:2:1 ratio of 215 F_3 progenies of Hope × Ridit in reaction to bunt form L–4*

Item	Goodness of fit for indicated progenies			Total
	Resistant	Heterozygous	Susceptible	
Observed (m+x)	66	100	49	215
Calculated (m)	53.75	107.5	53.75	215
$\frac{x^2}{m}$	2.792	.523	.420	3.735

P=0.16.

Mention already has been made of the difficulty in determining accurately the presence or absence of dominance. In the Hope × White Odessa cross the number of factor pairs involved in reaction to form L–4 can not be ascertained. However, the dominance or partial dominance of susceptibility is suggested from the distribution of the F_3 progenies (fig. 2, A).

In the Hope × Ridit cross a single-factor difference is indicated. The presence or absence of dominance could be readily ascertained from a comparison of the reaction of F_1 plants with that of the parents, but seeds for this test were not available. Nor can an accurate determination be made from the F_3 progenies because of the high variability in the parental rows resulting from the small number of plants per row. However, the distribution of F_3 progenies in Figure 2, B, suggests the absence of dominance of either resistance or susceptibility.

RELATIONSHIP OF BUNT REACTION TO OTHER CHARACTERS

In the F_3 progenies of all three crosses, data were obtained on length of awn, color of glume, and winter or spring habit of growth, wherever these characters differentiate the parents. An attempt was made to discover, by means of the correlation-ratio method, any relationship in inheritance between bunt reaction and these three characters. In no instance was there more than a suggestion of any such association.

SUMMARY

Hope, a spring variety of wheat (*Triticum vulgare*) which, when sown in spring, is highly resistant to three physiologic forms of *Tilletia tritici* (T–1, T–2, T–3) and two forms of *T. levis* (L–4, L–5) but which, when sown in the fall, is susceptible to the five forms, was crossed with Jenkin, a spring variety of wheat (*Triticum compactum*) which is susceptible to the five forms.

The seed of each of 195 F_2 plants was divided into three parts; one part was inoculated with T–3, another with L–5, and the third with a mixture of the remaining three forms, T–1, T–2, and L–4.

The factors in Hope for resistance to any one of these five physiologic forms seem to be the same as those for resistance to any of the others.

The results can be explained satisfactorily on the basis of three main factors for resistance, with no indication of the dominance of either resistance or susceptibility.

Crosses were made between Hope and the winter wheats, White Odessa and Ridit. F_3 progenies were tested with L–4, to which White Odessa is moderately resistant and Ridit highly resistant; one portion of each progeny was sown in the fall, another portion in the spring.

The spring-sown series showed scarcely a trace of bunt, indicating that the winter wheats, White Odessa and Ridit, carry at least some of the same factors as Hope for resistance to L–4 in spring sowing.

In the fall-sown series, the number of factors for reaction to L–4 in the Hope × White Odessa cross could not be determined. Apparently the reaction of Hope to this form, in fall sowing, differs from that of Ridit by a single main factor.

There is no evidence in the three crosses of linkage between the factor or factors for reaction to bunt and the factors for length of awn, color of glume, and winter or spring habit of growth.

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GROWTH OF THE COTTON ROOT-ROT FUNGUS IN SYNTHETIC MEDIA, AND THE TOXIC EFFECT OF AMMONIA ON THE FUNGUS[1]

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INTRODUCTION

The cotton root-rot disease, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, has been the subject of extended investigations since its discovery by Pammel (*5*)[2] in Texas in 1888. Although much knowledge has been obtained in regard to the ecology of the fungus, its life history, morphology, and some of its physiological aspects, there is as yet little known regarding the growth behavior of the pathogene under different conditions of nutrition. Several investigators have reported that the fungus grows well on a variety of media, such as cooked vegetables, various agars, plant decoctions, and sterilized roots or stems of many plants; but few attempts have been made to determine the effect of specific compounds in synthetic media on the growth of the fungus. One exception is the recent work of Ezekiel, Taubenhaus, and Fudge (*1*), who find that the fungus "grows readily in synthetic media" and is able to utilize both organic and inorganic nitrogen.

During the spring of 1931 the writers also studied the growth behavior of the fungus in nutrient solutions, with special attention to the nutrition of the organism with various nitrogen compounds. Comparisons were made of five different inorganic sources of nitrogen. In these studies it was found that practically no growth of the fungus occurred with ammonium nitrate or ammonium sulphate after 11, 18, and 31 days, whereas with calcium, sodium, and potassium nitrates growth was abundant. When this apparently toxic effect of ammonium salts was observed, other experiments were made to determine the effects of ammonium hydroxide and ammonia on the mycelial and sclerotial stages of the fungus. These experiments and observations form the basis of this paper.

MATERIALS AND METHODS

Duggar's solution for fungi was employed for the nitrogen comparisons, and the concentration of chemicals in the solution was as follows: M/4 dextrose, M/20 KH_2PO_4, M/100 $MgSO_4$, and a trace of $FePO_4$. To this was added M/5 KNO_3 or the other nitrogen carriers having an equivalent amount of nitrogen and calculated to yield approximately 12.4 g per liter. For obtaining these dilutions the following stock solutions were prepared: M/2 dextrose, M/4 KH_2PO_4, M/10 $MgSO_4$, M/1000 $FePO_4$, M/1 KNO_3, M/2 $Ca(NO_3)_2$, M/.582 $(NH_4)_2SO_4$, and M/1.29 NH_4NO_3. The cultures were grown in 125-cc Erlenmeyer flasks containing 50 cc of medium. They were prepared in triplicate

[1] Received for publication Nov. 16, 1932; issued August, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 117.

and given fractional sterilization (100° C. for one half hour for 3 days).

Owing to the fact that the fungus did not grow readily in the liquid medium when the inoculum was submerged, a hanging basket made of no. 24-gage nichrome wire was suspended in each flask to support the inoculum on the surface of the medium. By such a method, growth of the fungus may be readily obtained on any suitable nutrient solution. The flasks were inoculated after sterilization in the usual manner with a 14-day-old carrot-agar culture of the fungus, and care was taken to introduce approximately the same quantity of inoculum into each flask.

Hydrogen-ion determinations of uninoculated media in each series of cultures were made immediately after sterilization, and the dry weight of the fungous mats and the pH values of the filtrates were recorded at designated intervals, namely, 11, 18, and 31 days. At the intervals indicated the mats were collected on filter paper by means of a Büchner filter, dried to constant weight in a Freas oven, cooled for a uniform period in a calcium chloride desiccator, and weighed. The pH values were determined colorimetrically by Hellige disk comparators. The results are given in table 1.

TABLE 1.—*Weight of mycelium of Phymatotrichum omnivorum and pH of filtrates after 11, 18, and 31 days' growth in Duggar's solution containing different nitrogen equivalents*

Source of nitrogen added to Duggar's solution	Average [a] dry weight of mats, including dry weight of inoculum [b], after—			pH value of uninoculated solution after sterilization	pH value of filtrates after—		
	11 days [c]	18 days	31 days		11 days	18 days	31 days
	Mg	*Mg*	*Mg*				
KNO_3	282	574	451	4.3	5.3	6.0	5.8
$NaNO_3$	172	651	743	4.0	5.1	5.7	5.9
$Ca(NO_3)_2$	872	974	620	3.8	4.6	4.6	5.1
NH_4NO_3	84	84	153	3.9	4.4	4.4	4.3
$(NH_4)_2SO_4$	90	89	171	4.0	4.4	4.4	4.4

[a] Averages of 3 cultures.
[b] Average dry weight of inoculum, 87 mg.
[c] One culture only.

EFFECT OF NITROGEN FROM DIFFERENT COMPOUNDS ON GROWTH OF THE FUNGUS

It will be noted from table 1 that calcium nitrate when supplied as the nitrogen source produced the best fungous growth, which reached a maximum at the end of 18 days and declined after a longer interval, with a tendency toward degeneration of the mycelium. The next best results were obtained with sodium nitrate and with potassium nitrate. In the cultures containing the ammonium salts as nitrogen carriers no growth had occurred after 18 days, and there was only very slight evidence of mycelial growth on the inoculum in these after 31 days, when the experiment was terminated. The effects of the different nitrogen carriers on growth of the fungus in these studies are shown graphically in figure 1.

In the presence of calcium nitrate the fungus not only produced an abundant mat growth, but strand hyphae also developed above

the substratum along the walls of the flasks. In the potassium and sodium group, however, the mats were more compact, with strand hyphae almost entirely absent, and a tendency toward subsurface growth (fig. 2).

With the exception of the cultures containing ammonium salts, the filtrates became less acid as growth of the fungus progressed.

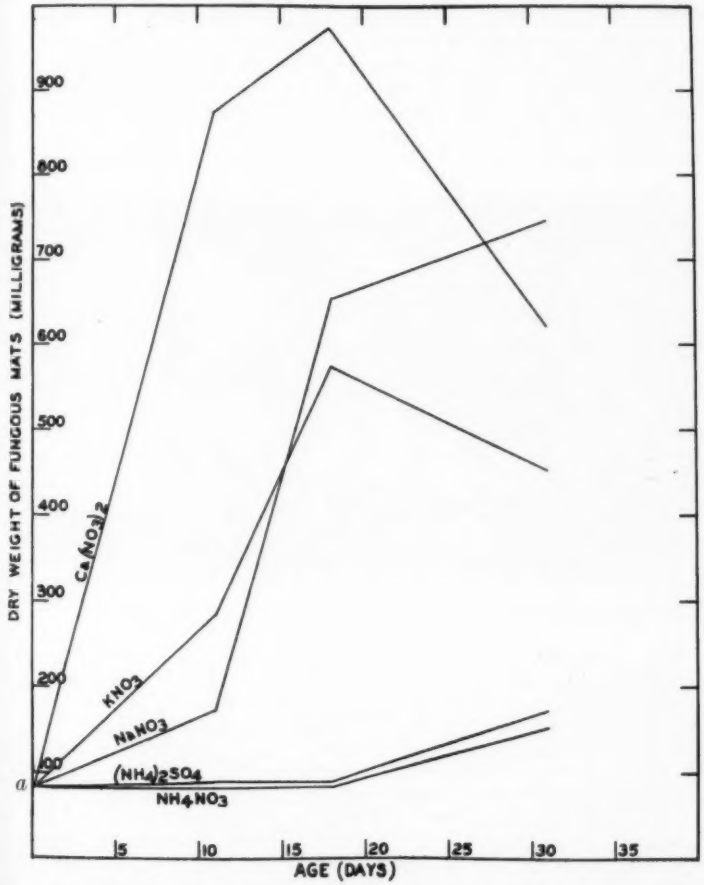

FIGURE 1.—Dry weights of *Phymatotrichum omnivorum* mats obtained in Duggar's solution with different nitrogen equivalents after 11, 18, and 31 days; *a*, dry weight of inoculum.

The tendency toward alkalinity in the cultures might be explained by the fact that certain fungi, unlike many bacteria, do not produce acid from carbohydrates. Waksman and Joffe (7) found that cultures of actinomycetes, when grown on $NaNO_3$ with the carbon source varied, showed a tendency toward alkalinity. His explanation was that the nitrate, under the influence of these organisms, is reduced to nitrite, and the liberated oxygen is united with the reducing hydrogen to form hydroxyl ions which reduce the hydrion of the media.

These data indicate, therefore, that the lack of growth of the fungus in these cultures was due to the direct toxic effect of the ammonia. This has been determined by further experiments which will now be discussed.

EFFECT OF AMMONIUM HYDROXIDE AND AMMONIA ON GROWTH OF THE MYCELIUM

The procedure adopted for studying the apparently toxic effect of ammonia on the fungus was as follows: Fresh cultures were prepared in 125-cc Erlenmeyer flasks containing 50 cc of neutral carrot agar, and after growth was well established over the medium the mycelium in each of four flasks was exposed for 20 minutes to dilute ammonium hydroxide (0.1, 0.5, and 1 percent by volume, prepared from 28 percent ammonia water) by pouring 50 cc of the solution into each

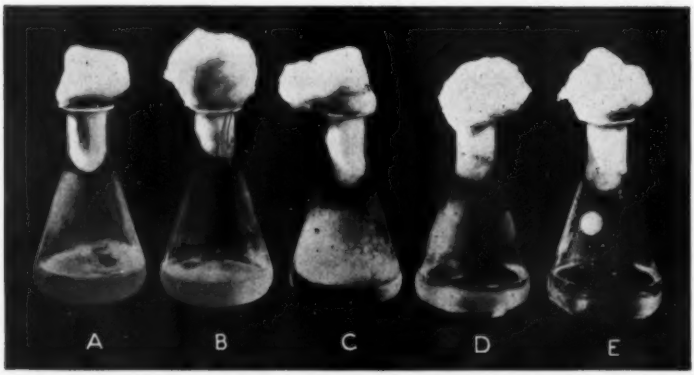

FIGURE 2.—Growth of *Phymatotrichum omnivorum* in Duggar's solution with different sources of nitrogen: A, Sodium nitrate; B, potassium nitrate; C, calcium nitrate; D, ammonium nitrate; E, ammonium sulphate. Photographed after 18 days' growth.

culture. Four untreated flask cultures were reserved as checks. After the above-mentioned exposures, sectional agar blocks containing the mycelium were removed from the treated and check flasks, washed thoroughly in sterile distilled water and transferred immediately to neutral carrot-agar slants in test tubes. In every case the inoculum from the cultures treated with ammonium hydroxide failed to grow, but growth occurred in all cases from the check inoculum.

Other flask cultures were subjected to ammonia treatment for 30 seconds by generating the gas from 500 cc of 28 percent ammonia water and allowing this to enter the culture flask by means of glass tubing. This treatment also completely inhibited growth of the fungus when transfers were made. The effect of exposing the fungus to 0.1 percent ammonium hydroxide for 20 minutes and to NH_3 gas for 30 seconds is shown in figure 3.

EFFECT OF AMMONIA ON GERMINATION OF SCLEROTIA

Tests were made with root-rot sclerotia by exposing them to ammonia for short intervals (10, 15, and 20 seconds). In these tests 200 cc of 28 percent ammonia water was boiled, and the gas thus generated was allowed to enter Petri dishes containing sclerotia.

Ten sclerotia were used for each experiment, and the same number, exposed for the same intervals in sterile water, were reserved as checks. Immediately after exposure the sclerotia were transferred aseptically to neutral carrot agar and incubated at 29° C. Examinations for viability were made at intervals of 48 and 72 hours. The ammonia-treated sclerotia failed to germinate in any of the tests, but the viability of the checks was 100 percent.

The effect of ammonium hydroxide on the germination of sclerotia was also studied under laboratory conditions to determine the strength that would probably be effective for application in the field. In these experiments individual sclerotia of average size were exposed from 1 to 20 minutes in concentrations of ammonium hydroxide ranging from 0.5 to 3 percent. Twenty sclerotia were used for each exposure, and the same number, exposed for the same interval in sterile water

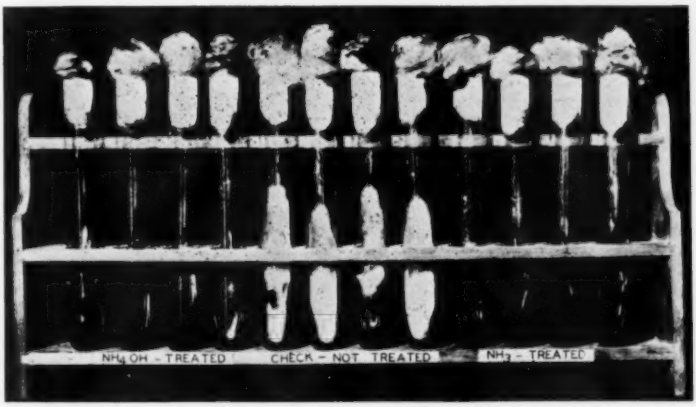

FIGURE 3.—Seven-day-old transfers of *Phymatotrichum omnivorum* cultures subjected to treatment with ammonium hydroxide for 20 minutes and with ammonia for 30 seconds, respectively.

only, were used as checks. The sclerotia had formed in type-soil cultures 104 days old. Immediately after exposure the sclerotia were washed in sterile distilled water, transferred to neutral carrot agar in Petri dishes, and incubated for 68 hours at 29° C. The germination results were then recorded and are given in table 2.

TABLE 2.—*Effect of ammonium hydroxide on viability [a] of Phymatotrichum omnivorum sclerotia*

Concentration of NH_4OH	Number of sclerotia [b] viable after exposure for—			
	1 minute	5 minutes	10 minutes	20 minutes
0.5 percent	13	2	[c] 1	0
1.0 percent	8	0	0	0
2.0 percent	0	0	0	0
3.0 percent	0	0	0	0
Check [d]	20	17	19	17

[a] Viability readings made after 68 hours' incubation.
[b] Number of sclerotia treated, 20.
[c] Sclerotial mass.
[d] Exposed in sterile water.

It is observed from these data that a 1 percent solution of ammonium hydroxide inhibited germination of sclerotia after an exposure of 5 minutes. The results obtained are comparable with those reported by King, Loomis, and Hope (*3*), who exposed sclerotia in 1 percent formalin solutions for 30 minutes and noted a reduction in germination, though some of the sclerotia survived.

EFFECT OF AMMONIA ON MYCELIUM IN INFECTED COTTON ROOTS

LABORATORY EXPERIMENTS

During the latter part of August several large root-rot-infected cotton plants were collected in the field and removed to the laboratory for chemical treatment. The tops were cut off and discarded, and the infected roots were washed thoroughly in tap water to remove the soil particles. After the surplus moisture had been removed with filter paper, the roots from each plant were placed in 500-cc Erlenmeyer flasks in duplicate and exposed to ammonia for 30 seconds and 1 and 2 minutes, respectively, the gas being generated in the manner previously described. Immediately after exposure the roots were placed in moist chambers and tested for viability of the fungus at 29° C. The ammonia treatments prevented growth after an exposure of 1 minute; good growth occurred in all the checks.

In this connection, King, Loomis, and Hope (*3*) studied the effect of formaldehyde gas on root-rot strands growing on moist, sterile sand in glass tubes, and found that the elongation of the strands in the tubes ceased soon after they were exposed to the gas liberated from a 1½ percent formalin solution. They report further that "root-rot sclerotia, exposed strands, and active mycelium on cotton root tissues, placed in glass tubes filled with sand, were killed when exposed for 21 hours to gas liberated from a 1½ percent formalin solution."

FIELD EXPERIMENTS

Field tests were conducted at various times during the season to determine the effectiveness of ammonium hydroxide when applied in the soil to the roots of infected cotton plants. In a preliminary test 4 percent solutions of this disinfectant did not completely inhibit growth of the fungus, consequently 6 percent solutions were used in subsequent experiments. Large mature freshly wilted plants, representing primary centers of infection, were selected and treated on August 25 with ammonium hydroxide by saturating the soil about the roots at the rate of 1 gallon per plant. Two plants were used for each test and a like number for the checks. After 48 hours' exposure the plants were removed from the soil, the roots cut off, washed, and placed in moist chambers at room temperature to test the viability of the mycelium. Observations on growth of the fungus from the tissues of the roots were made at 24-hour, 48-hour, and 5-day intervals. No growth developed from the roots of the treated plants, whereas copious development of root-rot mycelium was observed on the roots of the checks after 48 hours' incubation.

The experiment was repeated on September 22, and on this date 6 additional plants were treated with 6 percent NH_4OH as previously described and 6 were tagged as checks. The roots were placed in

moist chambers 40 hours after treatment, and growth observations were recorded after 48 hours and 5 days. With one exception, the mycelium was killed in the tissues of the roots of plants treated with

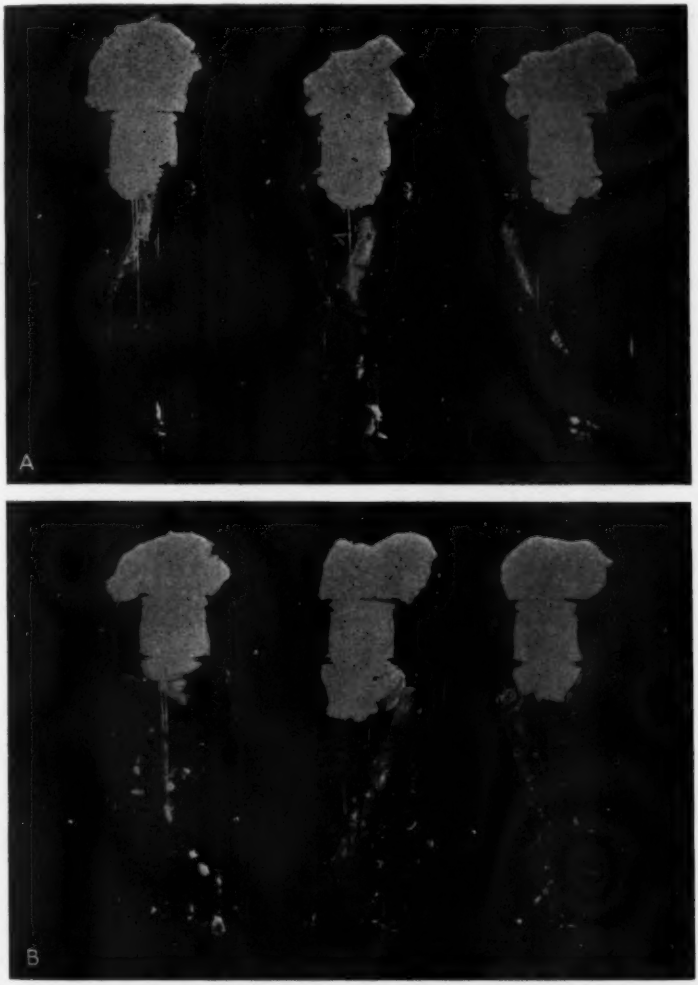

FIGURE 4.—Effect of field applications of ammonium hydroxide on roots of cotton plants infected with *Phymatotrichum omnivorum* after being in moist chamber at 29° C. for 5 days: A, Roots from infected plants treated for 48 hours with 6 percent ammonium hydroxide. Note absence of *Phymatotrichum* mycelium. B, Check, roots from infected plants.

6 percent ammonium hydroxide (fig. 4), and in the single case where growth actually developed it was noticeable only to a slight extent after the maximum period of incubation.

EFFECT OF AMMONIUM HYDROXIDE ON COTTON PLANTS

In the preliminary tests with ammonia, used in disinfecting the roots of infected cotton plants in the field, it was observed that in the majority of cases 4 percent solutions of the hydroxide did not kill the plants. On the other hand, 2 percent solutions of formalin frequently caused wilting in 3 or 4 days, and in many instances considerable defoliation resulted.

In order to gain further information as to what concentrations of the ammonia water would be likely to prove sufficient to kill cotton plants under field conditions, and at the same time serve as a guide for experimental application for various ornamental plants, additional experiments were made. Plants were treated during August, September, and October 1931, with 4, 6, and 8 percent solutions, respectively, of ammonium hydroxide by saturating the soil, as previously indicated, about the roots at the rate of 1 gallon per plant. Afterwards observations were made each day for several weeks. The plants treated with 6 percent solutions survived the treatment (fig. 5), notwithstanding the fact that considerable injury resulted in the cortical and cambium tissues of the roots (fig. 6). The plants that received 8 percent solutions, however, were noticeably affected; 50 percent being killed within 7 to 9 days after treatment (fig. 5).

DISCUSSION

In interpreting the toxicity of ammoniacal nitrogen in the case of higher plants, other workers (*4*, *6*) have advanced the hypothesis of physiological acidity or increasing hydrogen-ion concentration as a partial explanation. However, the marked suppression of growth of the fungus in the presence of the ammonium compounds in these experiments does not appear to be attributable to the acid reaction of the solutions, inasmuch as the other nitrogen carriers in the uninoculated flasks also revealed considerable acidity, as is indicated by the pH values after sterilization.

The effect of ammonia in preventing growth of the fungus, as indicated in the experiments reported in this paper, may explain some of the discrepancies encountered in earlier field applications of manure for the control of root rot. In some cases root-rot losses have been definitely prevented by continued applications of barnyard manure (*2*), whereas in other cases no restriction of root rot has resulted. The effect of manure upon the root rot may depend upon the early or ammoniacal decomposition of the manure; this would be in accord with field experiments in which applications of ammonia killed the root-rot mycelium but did not kill the cotton plants.

The possibility of utilizing ammonia or ammonium-yielding compounds for control of the disease in cotton fields as well as for protecting ornamental plants or shade trees is suggested, and field experiments to determine to what extent the ammonium treatment can be applied to general farm practice are now in progress.

SUMMARY

The cotton root-rot fungus, *Phymatotrichum omnivorum*, was grown in Duggar's solution for fungi, and growth comparisons were made of five inorganic sources of nitrogen.

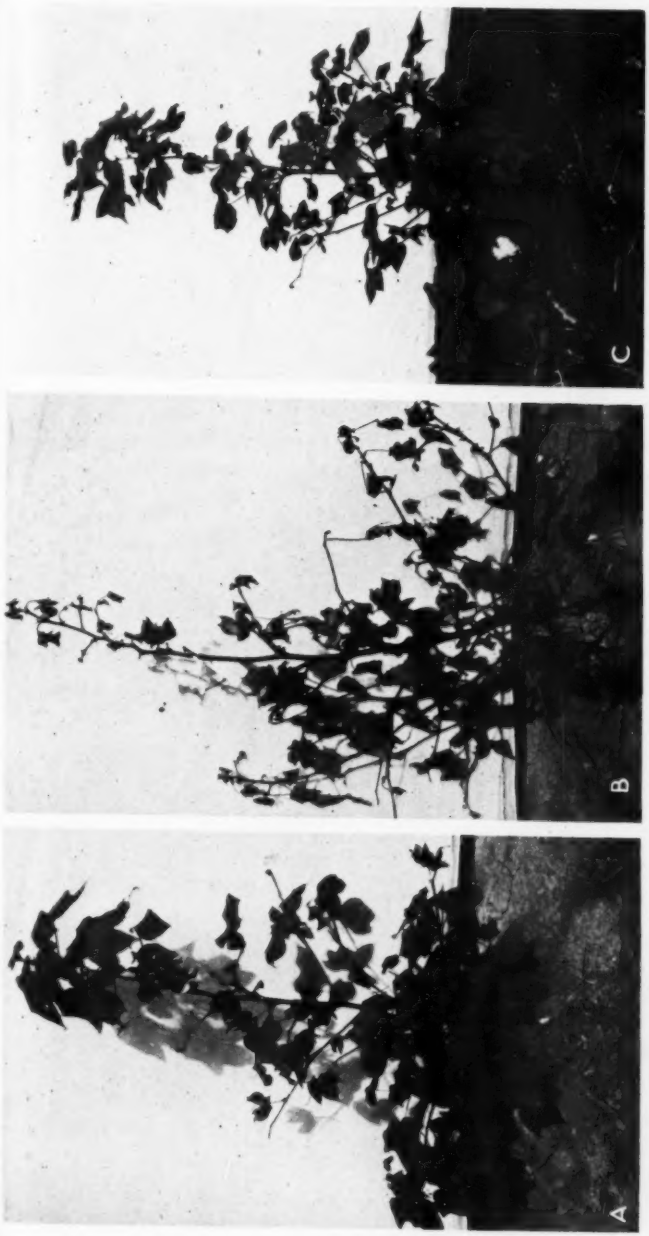

FIGURE 5.—Lone Star cotton plants after treatment with ammonium hydroxide, 1 gallon per plant: A, 6 percent, photographed after 60 days; B, 8 percent, photographed after 9 days; C, check.

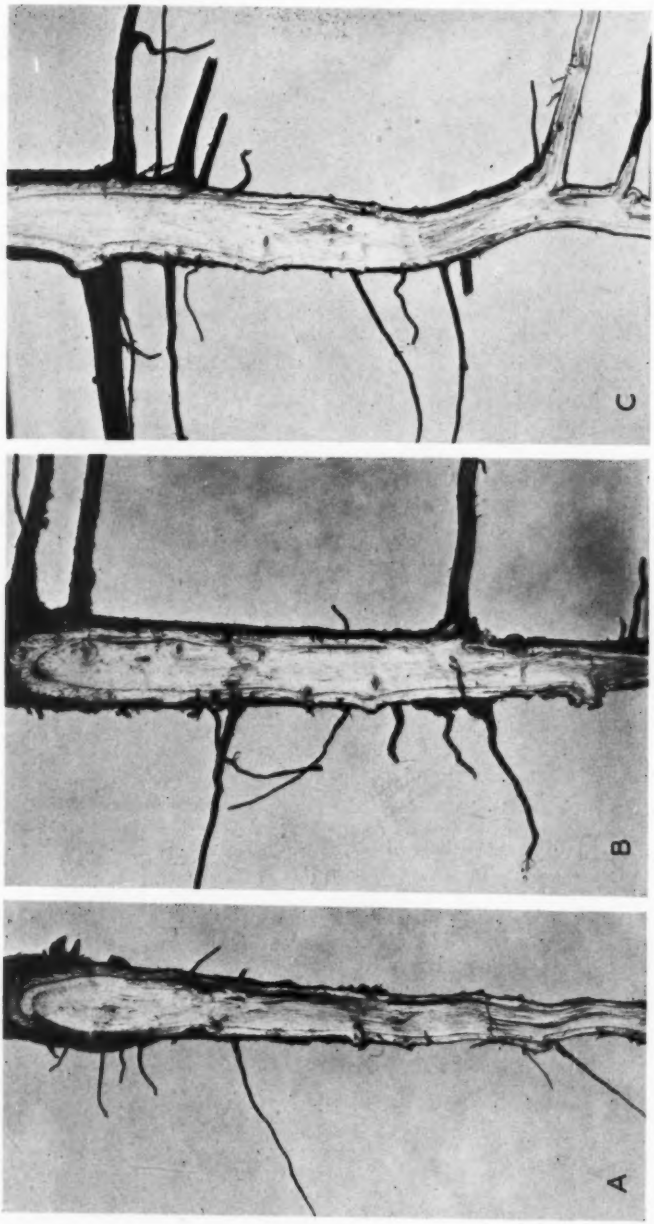

FIGURE 6.—Roots of Lone Star cotton plants after treatment with ammonium hydroxide, 1 gallon per plant: A, 6 percent, photographed after 60 days; B, 8 percent, photographed after 9 days; C, check.

With the nitrogen equivalents used at a concentration to yield approximately 12.4 g of nitrogen per liter, very little growth of the mycelium appeared after intervals of 11, 18, and 31 days with ammonium nitrate or ammonium sulphate, whereas with calcium nitrate, sodium nitrate, and potassium nitrate, abundant growth was produced.

Growth characters of the fungus obtained with the various nitrogen sources are described, and the toxic action of ammonium compounds is discussed.

With the exception of the cultures supplied with ammoniacal nitrogen, pH exponents of the filtrates increased as growth of the fungus progressed. The probable cause of the shifting of hydrogen-ion concentration of the media is given.

The apparent toxic effect of ammonia on the fungus was confirmed by subsequent tests, the mycelium being killed with ammonium hydroxide at a concentration as low as 500 parts per million after an exposure of 20 minutes. Gas liberated from 28 percent ammonia water also killed the mycelium in 30 seconds.

Ammonia inhibited germination of root-rot sclerotia after exposures as short as 10, 15, and 20 seconds. Sclerotia were killed in 5 minutes by 1 percent solutions of ammonium hydroxide.

Growth of the mycelium from root tissues of infected cotton plants was prevented by exposure to ammonia for 1 minute in laboratory tests. In field tests 6 percent solutions of ammonium hydroxide applied to the soil around the roots of infected cotton plants killed the mycelium in most cases, whereas abundant growth developed from the checks.

In experiments conducted in the field to determine the effect of ammonium hydroxide on cotton plants, it was found that mature plants were not killed with 4 or 6 percent solutions, although the cortical and cambium tissues of the roots were injured. The lethal effect of 8 percent solutions, however, was pronounced.

The inhibiting effect of ammonia on growth of the fungus and the probable relation this may have to actual control of the disease through continued applications of barnyard manure are discussed.

The possible utilization of ammonia or ammonium compounds for the control of the disease in cotton fields and for protecting ornamental plants is suggested by the experiments reported.

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DISTRIBUTION AND RATE OF FALL OF CONIFER SEEDS [1]

By HOWARD W. SIGGINS [2]

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INTRODUCTION

The first concern of American silviculture in its present stage of development is to secure satisfactory stands of young trees to take the place of mature trees which have been cut or are about to be cut. Where reliance is placed upon natural regeneration, the seeding habits of the species involved are matters of fundamental importance. For species which depend upon the wind for transportation of their seed from the parent tree to the place where the young tree is to grow, an understanding of the factors governing wind distribution of seeds would appear to be essential. The majority of the most important timber species of the world bear seeds adapted to wind dissemination, but a search of forestry literature antedating 1925 fails to bring to light any material of definite value on this subject. Most of the observations which have been made (*1, 3, 4*)[3] seem to have been based on studies of existing reproduction, accompanied by estimates (or guesses) as to the probable source of seed supply. In the few cases where the seed source was definitely known the meteorological side of the problem has remained untouched. No attempt appears to have been made to measure the rates of fall of each species of seed and thus determine how far it will be carried by winds of given velocities. Since 1925, however, several popular and semipopular articles in trade journals and other publications (*2, 6, 7, 13, 16*) have touched on other phases of the subject, particularly several written by Isaac (*10, 11, 12*), who began the study of this problem about the same time as the writer.

SEED CHARACTERISTICS WHICH AFFECT THE RATE OF FALL

Seeds which are adapted to dissemination by wind vary greatly in size, weight, and shape, in area and conformation of wing, and in combinations of these seed and wing qualities. In general one may say that species producing heavy seeds tend to have a faster rate of fall than species producing light seeds. However, weight alone is not the determining factor (*14, 15*). This is shown by the fact that seeds of *Chamaecyparis thyoides* (L.) B.S.P. averaging 0.0016 g in weight fall more rapidly than do seeds of *Pinus lambertiana* Dougl. averaging 0.293 g. Size of wing alone is no more valuable as a measure of rate of fall. Seed of *Abies amabilis* (Loud.) Forb. falls nearly twice as fast as seed of *Tsuga heterophylla* (Raf.) Sarg., though the former has by far the greater wing area.

[1] Received for publication Oct. 17, 1932; issued August 1933.

[2] In the original manuscript, prepared by Mr. Siggins just before his death in September 1929, he expressed grateful acknowledgments to the many persons who assisted him in this study by helpful suggestions or by collection of materials, and especially to Woodbridge Metcalf, associate professor of the University of California and extension forester of the State of California, with whose advice and assistance the study was begun in 1925.

[3] Reference is made by number (italic), to Literature Cited, p. 127.

A falling seed usually whirls around its center of gravity. This whirling is caused by the curvature of the wing. Since the amount of curvature varies greatly within any one species, the rate of whirling also varies (fig. 1). A few seeds of each species seem to have straight

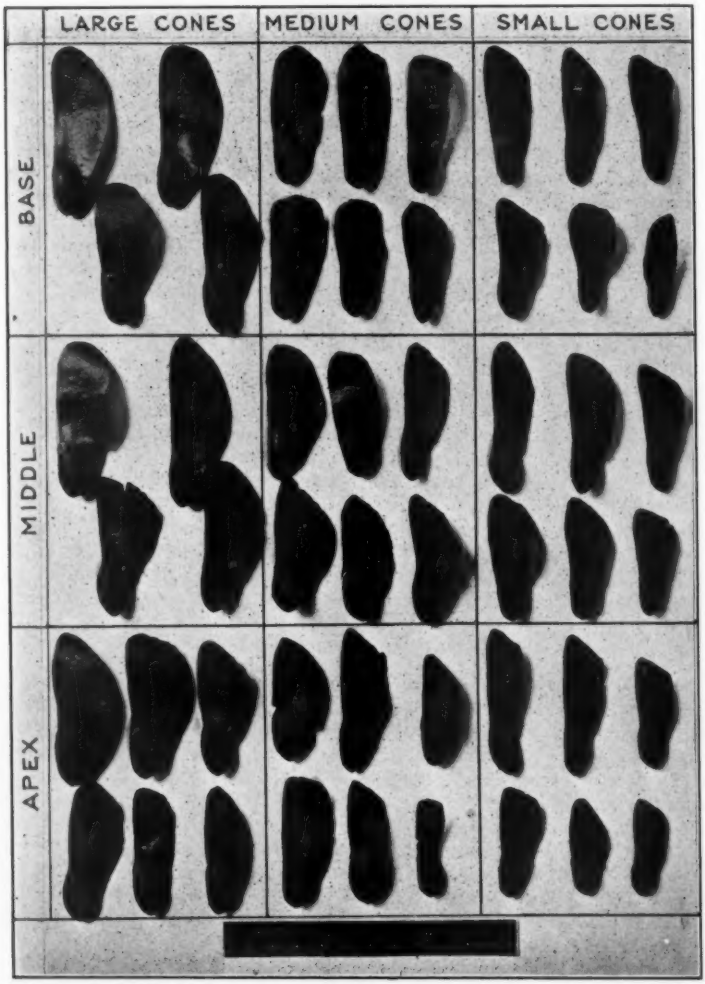

FIGURE 1.—Variability of sugar pine seeds from different cones and from different parts of each cone. Seeds are arranged vertically by size of cone: Left, large cones; middle, medium cones; right, small cones. Horizontally by location in cone: Top, base of cone; middle, midcone; bottom, apex of cone.

wings which do not produce a whirling motion. Such seeds fall very rapidly.

No clear-cut classification of seeds according to characteristics of wind resistance seems to be possible. From actual observation, however, one may say that—

(1) Seeds with marginal wings, such as redwood or birch, fall very rapidly for their weight. In these seeds the center of gravity corresponds closely to the center of surface area.

(2) Seeds with short broad terminal wings, such as the firs, have a less rapid rate of fall for their weight. In these seeds the center of surface area is somewhat removed from the center of gravity, though not far from the wingward end of the long seed.

(3) Seeds with terminal wings much longer than their width, such as the pines and spruces, fall least rapidly for their weight. In these seeds the center of surface area is considerably removed from the center of gravity.

From these observations one might advance the hypothesis that the efficiency of a seed wing, in supporting a given weight, is proportional to the distance between the center of gravity and the center of surface area (fig. 2). Since the seed whirls about its center of gravity, this distance becomes the radius of the circle described by the center of surface area. Inasmuch as the circumference is proportional to the radius, it might be said that the efficiency of the wing in supporting a given weight is proportional to the distance traveled by the center of wing area. Such a statement involves another factor, however, namely, the number of times the wing whirls about the seed in falling a given distance. A long and detailed study would be necessary to prove this hypothesis. No attempt was made to do so, because the only results of practical value have been more simply attained by measuring the average rate of fall for each species.

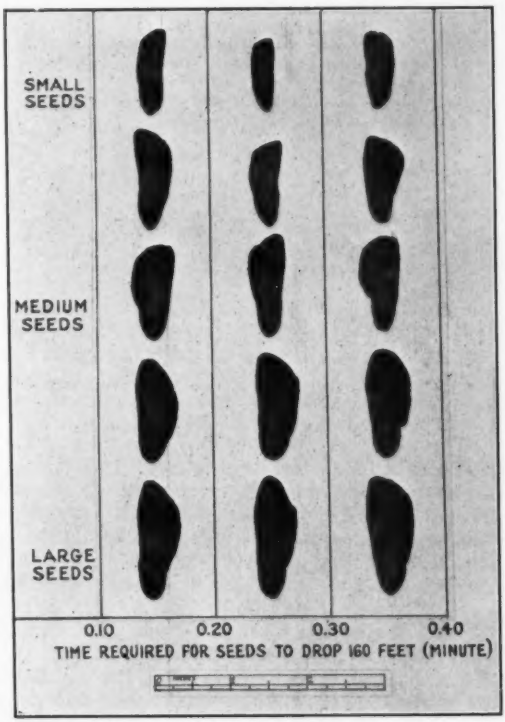

FIGURE 2.—Relation of seed and wing size in sugar-pine seeds having equal rate of fall.

MEASUREMENT OF RATE OF FALL

The first attempts to determine rates of fall in 1925 were made by liberating seeds from an elevation of 100 feet on a tall flagpole on the military parade ground of the University of California. The results obtained were highly variable. The next step was to release toy balloons with the seeds in order to be able to follow the flight more

easily. A given balloon should naturally have a constant rate of fall in quiet air. The balloons released with the seeds, however, showed the same variations in rate of fall as did the seeds. Furthermore, it was observed that the balloons sometimes followed an irregular line in descending. The solution was now apparent. Drilling students had trampled out the grass surrounding the flagpole and in several other spots. Convectional air currents, rising over the sun-warmed bare soil and descending over the large patches of cool sod, were affecting the rates of fall. Variations in direction of the wind during the 98 tests made at this place resulted in wide variations in the air conditions to which the falling seeds were subjected. The extent of these variations is shown in table 1.

TABLE 1.—*Range of rates of fall (feet per second) in rapid and slower falling lots of seeds of different species, showing the effects of convectional currents and other turbulence*

Species	Most rapidly falling lot		Least rapidly falling lot	
	First seed	Last seed	First seed	Last seed
Sugar pine	18.5	4.4	6.7	2.0
Redwood [a]	11.1	6.7	9.3	4.8
Ponderosa pine	8.3	3.5	3.1	1.2
White fir	11.1	5.6	4.8	1.1
Red fir	9.3	3.5	8.3	2.6
Grand fir	16.7	9.3	3.3	2.4
Sitka spruce	3.7	1.8	(b)	(b)
Douglas fir	5.6	2.7	2.8	.7
Incense cedar	8.3	2.8	(b)	(b)

[a] Redwood seeds are so small that they were impossible to follow in the air and very difficult to find in the grass. The maximum rates are probably too low.

[b] 6 lots of Sitka spruce and 1 lot of incense cedar were caught by ascending convectional currents and carried upward out of sight.

The next step was to find a place in which to conduct trials in undisturbed air. An ideal laboratory for this purpose was at hand in the campanile, or bell tower, on the University of California campus. This structure is a masonry shaft 302 feet high and 33 feet square at the base. It is divided into compartments by floors, the first at 30 feet from the ground and the successive floors every 25 feet above. In the center of the tower is a concrete elevator shaft, about 7 feet square inside. This runs from a pit 10 feet below the ground floor to the landing at the 180-foot level. The openings to the elevator at each floor are closed with solid oak doors. With all doors closed the shaft becomes a well-insulated chamber in which temperature conditions change very slowly and no air movement is detectable with a Biram anemometer.

The seeds were dropped from a trap held in the center of the shaft at the next-to-top landing and were caught on muslin screens laid on a concrete pier 5 feet high in the center of the pit. This gave a clear fall of 160 feet. The screens had to be limited in size and number because of the crowded conditions in the pit and the necessity for quick and certain manipulation. Ten screens 3 feet square were used. The first screen was in position when the releasing trap was opened. A second screen was laid on top of the first when the first whirling seeds appeared. Thereafter additional screens were superimposed every 0.1 minute. One man measured the time with a stop watch while a second man manipulated the screens. Occasionally mechanical difficulties caused slight errors in the time at which screens were

laid down, but the error was rarely as much as 1 second. Except when the mode of the distribution occurred near the time of changing screens, this error did not greatly affect the calculated mean.

After the seeds of a lot had all fallen the sample collected on each screen was transferred to a paper bag for later analysis. Table 2 is a typical analysis of one lot of seeds.

TABLE 2.—*Distribution by rate of fall of one lot of loblolly pine seeds dropped 160 feet in quiet air*

Time of falling (minutes)	Total seeds		Good seeds *		Weight of good seeds	
					Total	Average
	Number	*Percent*	*Number*	*Percent*	*Grams*	*Grams*
0.10–0.20	13	1.7	10	1.8	0.32	0.032
0.20–0.30	5	.7	5	.9	.17	.034
0.30–0.40	3	.4	2	.3	.06	.03
0.40–0.50	4	.5	1	.2	.03	.03
0.50–0.60	96	12.6	76	13.3	2.40	.032
0.60–0.70	376	49.3	324	56.7	10.70	.033
0.70–0.80	200	26.2	122	21.4	4.06	.033
0.80–0.90	43	5.6	23	4.0	.75	.033
0.90–1.00	15	2.0	8	1.4	.26	.032
1.00–1.10	8	1.0	0	0	0	0
Total	763	100.0	571	100.0	18.75	.033

* 75 percent of the seeds are good. The average good seed falls 160 feet in 0.6567 minute, 244 feet in 1 minute, or 4.1 feet in 1 second.

Table 2 shows that some seeds descend much more rapidly and some much more slowly than the mean rate of fall. The great bulk of the seeds, however, fall in a rather concentrated group. This concentration about the mean rate of fall is typical of all lots of seeds. Different samples of seeds of the same lot show differences in the calculated mean rates of fall, but these differences are well within the limits of experimental error.

TABLE 3.—*Percentage distribution by rate of fall of Pinus taeda seeds from different trees and localities when dropped 160 feet in quiet air*

Time of falling (minutes)	Seeds from Virginia							Seeds from Texas
	Tree no. 2	Tree no. 3	Tree no. 1	Tree no. 10	Tree no. 11	Tree no. 5	Tree no. 12	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0–0.30	6.0	2.4	3.3	2.6	9.9	4.5	6.1	5.6
0.30–0.40	1.4	.8	1.0	.4	1.9	1.1	3.5	1.2
0.40–0.50	2.0	1.2	2.4	.2	1.5	1.4	6.3	48.9
0.50–0.60	3.8	7.0	2.9	13.3	11.0	45.9	58.8	28.1
0.60–0.70	14.4	40.6	59.3	56.7	36.0	31.8	22.0	10.6
0.70–0.80	48.1	43.9	21.8	21.4	33.2	10.5	2.5	3.3
0.80–0.90	20.6	4.1	6.3	4.0	5.8	4.1	.8	1.3
0.90–1.00	3.1	0	2.2	1.4	.7	.4	0	.8
1.00–1.10	.6	0	.8	0	0	.2	0	.2
1.10–1.20	0	0	0	0	0	.1	0	0
Average weight of good seeds	*Gram* 0.031	*Gram* 0.023	*Gram* 0.035	*Gram* 0.033	*Gram* 0.021	*Gram* 0.021	*Gram* 0.030	*Gram* 0.026
Average rate of fall	*Feet per second* 3.8	*Feet per second* 3.9	*Feet per second* 4.0	*Feet per second* 4.1	*Feet per second* 4.2	*Feet per second* 4.5	*Feet per second* 4.9	*Feet per second* 5.3

One group of tests calling for special mention is the series in which *Pinus taeda* seed was used. The Appalachian Forest Experiment Station sent seven lots of loblolly pine cones collected in Virginia, each lot containing cones from only one tree. The calculated rates of fall,

obtained from one test with each lot of seed, were 3.7, 3.9, 4, 4.1, 4.3, 4.4, and 4.9 feet per second. The mean rate was 4.2 and the median rate 4.1 feet per second. These figures show that the results of the rather limited number of tests here reported cannot be considered as exact and invariable quantities. Furthermore, a lot of loblolly cones sent by the Southern Forest Experiment Station from Texas showed a decidedly different rate of fall, 5.3 feet per second, suggesting that seeds of the same species may develop regional characteristics which require study. Table 3 shows the results of these tests in more detail.

There seems to be some evidence of slight negative correlation between weight of seed and rate of fall in the eight lots of the species shown in table 3. The fastest falling seeds are lighter than the average weight. The heaviest seeds fall more slowly than the average. The negative correlation shown by different lots of this species apparently is contradictory to the general tendency in the genus Pinus for the rate of fall to be positively correlated with the average weight of the seed, as indicated by the data in table 4.

TABLE 4.—*Average rate of fall and average weight of seed in 12 species of pine*

Species	Source	Average rate of fall	Average weight
		Feet per second	*Gram*
Pinus lambertiana Dougl.	California	8.7	0.293
Pinus jeffreyi "Oreg. Com."	do	7.25	.135
Pinus palustris Mill.	Louisiana	5.8	.120
Pinus ponderosa Laws.	California	5.0	.058
Pinus caribaea Mor.	Florida and Louisiana	4.6	.042
Pinus taeda L.	Virginia	4.2	.027
Pinus echinata Mill.	Louisiana and Texas	3.85	.012
Pinus balfouriana Murr.	California	3.7	.024
Pinus strobus L.	North (?) Carolina	3.3	.024
Pinus virginiana Mill.	do	2.85	.010
Pinus pungens Lamb.	do	2.8	.013
Pinus contorta Loud.	Wyoming	2.7	.0055

Seeds of other genera do not show the same correlation between weight and rate of fall as do seeds of the pines. Table 5 gives figures on some of the other species which have been tested.

TABLE 5.—*Average rate of fall and weight of seed of species other than pine*

Species	Source	Average rate of fall	Average weight of good seeds
		Feet per second	*Gram*
Abies grandis Lindl.	California	5.7	0.024
Abies concolor Lindl. and Gord.	do	5.7	.070
Abies magnifica Murr.	do	5.3	.110
Abies fraseri (Pursh) Poir.	North Carolina	5.1	.013
Abies amabilis (Loud.) Forb.	Oregon	4.95	.075
Betula lutea Michx.	North Carolina	5.1	.0013
Chamaecyparis thyoides (L.) B.S.P.	Virginia	9.6	.0016
Libocedrus decurrens Torr.	California	5.9	.065
Liriodendron tulipifera L.	North Carolina	6.2	.056
Picea rubra Link.	do	3.9	.0053
Picea sitchensis (Bong.) Carr.	California	3.1	.0034
Pseudotsuga taxifolia (Lam.) Britt.	do	4.4	.017
	Colorado	4.4	.009
Sequoia sempervirens (Lamb.) Endl.	California	8.6	.0074
Thuja plicata D. Don	Oregon	5.1	.0015
Tsuga heterophylla (Raf.) Sarg.	do	2.6	.0021

DISTRIBUTION OF SEEDS FROM THE CONE

Any group of seeds liberated from one tree at the same instant will tend to be distributed in a straight line to leeward of the tree. If all seeds produced by a tree were to be released at one time the result would be an undesirable concentration of seeds in a limited area and a total lack of seeds in other areas. The mechanisms of dissemination are such, however, as to provide a continuing supply of seeds over a considerable period (fig. 3). A pine cone, for example, opens one scale at a time. As the scale opens the two seeds which were borne under it are permitted to drop out. Ordinarily several days elapse between the escape of the first and last seeds from any one cone. Furthermore, the cones on different parts of the same tree do not all open at the same time. One tree may be casting seed for several weeks. Variations in direction and velocity of the wind during this period result in widespread scattering of the seeds. Where the winds are rather consistently from one direction the area seeded will be in the form of a V, with the apex at the base of the tree. The areas of the concentric radial zones increase as the squares of the radii. The greatest number of seeds per square foot of ground will therefore be found quite close to the base of the tree. The concentration of seeds will then decrease rather gradually with increasing distance from the tree until the point is reached where the average seed is dropped by the wind of average velocity. Beyond this point the concentration of seeds will diminish very rapidly.

In a virgin forest the supply of seed is so great and so uniformly distributed that there is no significance to this question of how far seeds are carried. However, it is important when considering the seeding of a cut-over area from a body of uncut timber to windward. The distance of seeding from the uncut timber is controlled by the velocity of the wind blowing at right angles to the edge of the cutting.

A forester who wishes to plan carefully the distribution of seed trees on a cut-over area must consider not only the velocity but also the variability of direction of winds which are to distribute the seeds. The ideal condition would exist when winds might be expected to blow from all directions. Each tree would then stand in the center of a circle and would seed the maximum area without overlapping. Where the prevailing winds come principally from one direction, however, the seed distribution will be in long narrow V's and considerable overlapping will be necessary to provide for completely covering the ground.

While the question of quantity of seed required might not seem to be pertinent, it may be well to mention the subject as a matter of caution. The destruction of seeds by birds and rodents is so great that a distribution of seed trees sufficient to cover the area with a satisfactory concentration of seeds might prove entirely inadequate for securing a good stand of reproduction. Taylor[4] reports that caged ground squirrels ate on an average 340 seeds of ponderosa pine in one night. Chipmunks ate 237 pine seeds in the same time. Four seed trees per acre, averaging 92,000 seeds per year, he says—

> would be insufficient to do more than win the grateful appreciation of the resident squirrels and chipmunks were it not for the young pines that actually do come in on these cut-over areas in defiance of biological statistics.

[4] No comprehensive report of the findings of Walter P. Taylor in the Southwest has been published.

FIGURE 3.—Progressive steps in the opening of a ponderosa pine cone.

These trees, of course, arise from seeds which have fallen where they escaped the search of the rodents and birds or from seeds which have been buried and forgotten by the little beasts.

Because of this destruction of the seed crop, many trained investigators have been willing to accept such statements as "seed trees should be so spaced that no tree would be depended on to seed to a distance greater than the height of the tree." It is now evident that at least a part of the reproduction which is found at long distances from any seed trees may safely be attributed to wind-blown seeds. In the case of large, severely burned areas, the bulk of the rodents are probably killed by the fires or die of starvation or disease shortly afterward. This renders the areas comparatively safe for the seeds which fall upon them, and the rate of establishment is high when soil and moisture conditions are favorable.

SUMMARY AND CONCLUSIONS

Each species of tree produces seeds having a characteristic average rate of fall, although this rate may vary considerably with the region in which the tree is grown.

Because of the range in rates of fall of seeds from one source and variations in the height at which the seeds are produced and in the direction and velocity of winds which distribute these seeds, it is not possible to predict exactly the distribution of seeds which will be obtained from a given seed tree in any one season. The total distribution over a period of years can be predicted with reasonable accuracy, however, if the meteorological conditions of the locality are carefully studied.

Forest-tree seeds adapted to dissemination by wind are carried to much greater distances than was previously supposed, but under normal conditions the dissemination of tree seeds to great distances is of small importance to the silviculturist.

Under certain unusual conditions the eventual reforestation of extensive burns may be assured by trees grown from seeds carried for many miles. Such seeds may number only one in a million, but the great number of seeds produced renders the aggregate of extreme importance in such cases.

The effective distribution of seeds is much smaller than the actual distribution because of the destruction of seeds by rodents. Rodent-control measures should greatly reduce the economic loss caused by the present necessity of tying up large amounts of lumber in seed trees. Until this is accomplished, the quantity of seed produced per acre, rather than the distance to which it is distributed, must be the criterion for determining the number of seed trees to be left for reforestation of cut-over lands.

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